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(54) Title: NOVEL ANTIBODIES FOR CONFERRING PASSIVE IMMUNITY AGAINST INFECTION BY A PATHOGEN IN HUMANS

(57) Abstract

Proteins and peptides derived from a murine *P. falciparum* monoclonal antibody, including synthetic humanized variable light chain and variable heavy chain sequences, CDR peptides, and humanized antibodies useful in therapeutic methods and compositions for conferring passive immunity to infection by a malaria-causing parasite are provided.

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NOVEL ANTIBODIES FOR CONFERRING PASSIVE IMMUNITY AGAINST INFECTION BY A PATHOGEN IN HUMANS

Field of the Invention

This invention relates generally to the field of monoclonal and recombinant antibodies directed to epitopes on selected pathogens, e.g., a malaria parasite, methods for preparing and using, and compositions employing, these antibodies.

Background of the Invention

Malaria is a severe and widespread disease, caused by various species of the protozoan parasite genus *Plasmodium*, including four species that infect man, e.g., *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* [See, e.g., V. Enea et al., Science, 225:628-630 (1984)]. Malaria remains one of the most widespread and fatal diseases in the world today because of the lack of an effective vaccine and programs to control vector populations, as well as new drug-resistant strains. Generally, treatment of malaria relies heavily on prophylactic drugs, such as the 4-aminoquinolines. However, for most cases, drug resistance by *P. falciparum* and the production of some undesirable side effects, have undermined the efficacy of these drug therapies.

The focus of much research effort in the field of malaria prophylaxis is the sporozoite form of the *Plasmodium* parasite, particularly the circumsporozoite (CS) protein [Clyde et al., Am. J. Trop. Med. Hyg., 24:397 (1975); Rieckman et al., Bull. WHO, 57(1):261 (1979); and U. S. Patent 4,957,869]. The cloning and characterization of the CS protein genes or fragments thereof of a number of *Plasmodium* species and recombinant expression thereof in *E. coli* or yeast host cells have been reported. The central repeat domain of the CS proteins is immunodominant, i.e., if one injects

sporozoites into an animal, the animal produces anti-repeat antibodies. The first anti-sporozoite candidate vaccine tested in man was based upon the repetitive epitopes found on the CS protein of *P. falciparum* consisting of $(AsnAlaAsnPro)_{37}(AsnValAspPro)_4$ [SEQ ID NO: 1], which is invariant in a number of strains examined to date. Clinical trials utilizing a vaccine candidate, called R32tet32, consisting of NH_2-Met-Asp-Pro-$[(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_{15}(Asn\text{-}Val\text{-}Asp\text{-}Pro)_1]_2$-Leu-Arg-Arg-Thr-His-Arg-Gly-Arg-His-His-Arg-Arg-His-Arg-Cys-Gly-Cys-Trp-Arg-Leu-Tyr-Arg-Arg-His-His-Arg-Trp-Gly-Arg-Ser-Gly-Ser-COOH [SEQ ID NO: 2], produced a protective response in a human volunteer against the sporozoite challenge [see, Ballou et al., The Lancet, June 6, 1987, pp. 1277-1281; and European Patent Publication No. 0192626, published August 27, 1986, incorporated herein by reference].

Numerous monoclonal antibodies (mAbs) directed toward proteins from various stages of the *Plasmodium* life cycle have been identified and shown to be effective in passive transfer experiments in mice and monkeys [Y. Charoenvit et al., J. Immunol., 146(3):1020-1025 (1990)]. However, the use of antibodies for the treatment or prophylaxis of malaria may have disadvantages. The administration of murine or other animal antibodies to humans may be limited by the adverse immune response of humans to the foreign antibody, e.g., rapid clearance and toxic side effects. Such immune responses in humans have been shown to be directed against both immunoglobulin constant and variable regions of murine antibodies.

Several techniques have been described which suggest alteration of murine (and other species) antibodies to reduce the occurrence of an immune response in a desired species, e.g., human, to the parent antibody [See, e.g., PCT Patent Publication No. PCT/WO86/01533, published March 13, 1986; British Patent Application No.

GB2188638A, published October 7, 1987; Amit et al., Science, 233:747-753 (1986); Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10033 (1989); PCT Patent Publication No. PCT/WO90/07861, published July 26, 1990; and Riechmann et al., Nature, 332:323-327 (1988)]. While the prior art suggests possible experimental techniques, none show how to provide the combination of properties required for effective prevention of *in vivo* growth of *P. falciparum*.

There remains a need in the art for alternative methods of providing immunity against infection with selected pathogens, e.g., a malarial parasite, particularly for a prophylactic agent capable of providing effective short-term protection.

Summary of the Invention

In one aspect, the present invention provides complementarity determining region (CDR) peptides from a monoclonal antibody directed against a selected epitope on a pathogen, as well as fragments and analogs of these peptides. Preferably, the antibody is capable of binding an epitope of *Plasmodium*, particularly the CS repeat region epitope or a fragment thereof, e.g., murine anti-*P. falciparum* mAb NFS2. These CDRs retain the antigen binding specificity of the mAb from which they were derived.

Another aspect provides an isolated, naturally occurring or synthetic, humanized immunoglobulin light or heavy chain variable region amino acid sequence comprising one or more CDR sequences originating from the light or heavy chain of such a selected antibody.

In yet a further aspect, the invention provides a fusion protein comprising a first amino acid sequence derived from the variable light chain and/or heavy chain of an anti-*Plasmodium* antibody, an anti-*Plasmodium* CDR, a

functional fragment or analog thereof. The first selected amino acid sequence is operatively linked or fused to a second selected amino acid sequence. These fusion proteins are characterized by the antigen binding specificity of the mAb from which the first selected amino acid sequence is derived.

A further aspect of the invention provides an engineered antibody with specificity for the selected *Plasmodium* epitope, e.g., *P. falciparum* repeat region.

In another aspect, the invention provides a *P. falciparum* antibody or fragment thereof produced by screening hybridoma products derived from any species immunoglobulin repertoires, or human or murine antibody combinatorial libraries, with the epitope of mAb NFS2.

In a further aspect, the present invention provides F_{ab} fragments of the above-described engineered antibodies or anti-*Plasmodium* mAbs.

As yet additional aspects, the invention provides nucleic acid sequences which encode the proteins, peptides, antibodies and fragments described herein, as well as plasmids containing one or more of the sequences, host cells transformed therewith, and methods for producing the products of expression of these nucleotide sequences in host cells, e.g., mammalian cells.

Other aspects provided by the invention include a pharmaceutical composition and a prophylactic method for conferring passive immunity to a human anticipating exposure to a malarial parasite, comprising an effective amount of at least one protein, antibody, peptide or fragment described herein and a pharmaceutically acceptable carrier or diluent.

Other aspects and advantages of the present invention are described further in the following detailed description of preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 illustrates the amino acid SEQ ID NO: 4 and nucleotide SEQ ID NO: 3 sequences of the naturally occurring light chain variable region of mAb NFS2.

Fig. 2 illustrates the amino acid SEQ ID NO: 6 and nucleotide SEQ ID NO: 5 sequences of a synthetic humanized light chain variable region Pfhzlc1-1 containing anti-*Plasmodium* CDRs SEQ ID NOs: 21-26. The CDRs are underlined.

Fig. 3 illustrates the amino acid SEQ ID NO: 8 and nucleotide SEQ ID NO: 7 sequences of synthetic humanized light chain variable region Pfhzlc1-2.

Fig. 4 illustrates the amino acid SEQ ID NO: 10 and nucleotide SEQ ID NO: 9 sequences of the naturally occurring heavy chain variable region of mAb NFS2.

Fig. 5 illustrates the amino acid SEQ ID NO: 12 and nucleotide SEQ ID NO: 11 sequences of a synthetic humanized heavy chain variable region Pfhzhc2-4.

Fig. 6 illustrates the amino acid SEQ ID NO: 14 and nucleotide SEQ ID NO: 13 sequences of synthetic humanized heavy chain variable region Pfhzhc2-3.

Fig. 7 is a schematic drawing of plasmid Pfhzhc2-3-Pcd employed to express a synthetic anti-*Plasmodium* heavy chain in mammalian cells. The plasmid contains a beta lactamase (Beta-lac) gene, an SV40 origin of replication (SV40), a cytomegalovirus promoter sequence (CMV), the synthetic heavy chain Pfhzhc2-3 SEQ ID NO: 13, a poly A signal from bovine growth hormone (BGH), a betaglobin promoter (beta glopro), a dihydrofolate reductase gene (DHFR), and another BGH sequence poly A signal in a pUC19 background.

Fig. 8 is a schematic drawing of plasmid Pfhzlc1-1-Pcn employed to express a synthetic light chain in mammalian cells. The plasmid differs from that of Fig. 7, in that it contains the synthetic humanized light

chain Pfhzlc1-1 SEQ ID NO: 5 rather than the heavy chain, and a neomycin gene (Neo) in place of DHFR.

Fig. 9 illustrates the nucleotide SEQ ID NO: 42 and amino acid SEQ ID NO: 43 sequences of a synthetic humanized heavy chain variable region Pfhzhc2-6.

Detailed Description of the Invention

The present invention provides prophylactic agents capable of conferring a short duration, protective immune state against infection of humans by selected pathogens in the immunized human, e.g., for epidemic control and for use by those anticipating exposure to the pathogen. Recombinant or engineered antibodies, preferably chimeric, humanized or human monoclonal antibodies, are capable of use as such passive protective proteins. These proteins in a prophylactic composition may be administered before anticipated exposure to the pathogen and would not require daily regimens of follow-up doses to mediate the short term protection.

While the following description refers specifically to antibodies capable of conferring passive protection to the sporozoite form of the pathogen, *P. falciparum*, a causative agent of malaria in humans, the invention described herein is not limited to any particular stage of that pathogen nor to that pathogen alone. The teachings of the present invention permit one skilled in the art to construct other recombinant antibodies directed to other selected pathogens, e.g., other species of *Plasmodium*, including the blood stages, liver stages, or gametocyte stages. Antibodies of the invention directed against the circumsporozoite CS gene of the other human infective parasites, e.g., *P. malariae*, *P. vivax* and *P. ovale*, may also be constructed according to this invention to provide passive transfer proteins useful against these parasitic infections.

Similarly, passive therapy agents prepared according to the invention may involve other infective agents, viruses, bacteria and the like. Additionally, such antibodies may also be useful as therapeutic agents for the treatment of acute stages of infections.

I. *Definitions*

"First fusion partner" refers to a nucleic acid sequence encoding an amino acid sequence, which can be all or part of an immunoglobulin heavy chain, a light chain, functional fragment thereof including the variable region from one or both chains and CDRs therefor, or an analog thereof, having the antigen binding specificity of a selected high titer antibody, preferably the murine antibody, NFS2.

"Second fusion partner" refers to another nucleotide sequence encoding a protein or peptide to which the first fusion partner is fused in frame or by means of an optional conventional linker sequence. Such second fusion partner is preferably heterologous to the first fusion partner. A second fusion partner may include a nucleic acid sequence encoding a second antibody region of interest, e.g., all or part of an appropriate human constant region or framework region.

"Fusion molecule" refers to the product of a first fusion partner operatively linked to a second fusion partner. "Operative linkage" of the fusion partners is defined as an association which permits expression of the antigen specificity of the anti-*P. falciparum* sequence (the first fusion partner) from the donor antibody as well as the desired characteristics of the second fusion partner. For example, a nucleic acid sequence encoding an amino acid linker may be optionally used, or linkage may be via fusion in frame to the second fusion partner.

"Fusion protein" refers to the protein encoded by the fusion molecule, which may be obtained by expression of the fusion molecule in a selected host cell. Such fusion proteins may be engineered antibodies, e.g., chimeric or humanized antibodies, or any of the antibody regions identified herein fused to immunoglobulin or non-immunoglobulin proteins and the like.

"Donor antibody" refers to an antibody (polyclonal, monoclonal or recombinant) which contributes its naturally-occurring or modified variable light and/or heavy chains, variable regions thereof, CDRs thereof or other functional fragments thereof to a first fusion partner, so as to provide the fusion molecule and fusion protein, with the antigenic specificity characteristic of the donor antibody. One donor antibody suitable for use in this invention is murine mAb NFS2 and others are described below.

"Acceptor antibody" refers to an antibody (polyclonal, monoclonal or recombinant) heterologous to the donor antibody, but homologous to the patient (human or other mammal) to be treated, which contributes all or any portion of the sequences of its variable heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to a second fusion partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of the heavy and light chains. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include functional fragments and analogs of the naturally occurring CDRs, which share or

retain the same antigen binding specificity as the donor antibody from which they were derived.

By "sharing the antigen binding specificity" it is meant, for example, that although mAb NFS2 may be characterized by a certain level of antigen affinity, and a CDR encoded by a nucleic acid sequence of NFS2 in an appropriate structural environment may have a lower affinity, it is expected that CDRs of NFS2 in such environments will nevertheless recognize the same epitope(s) as NFS2.

A "functional fragment" is a partial CDR sequence or partial heavy or light chain variable sequence which retains the same antigen binding specificity as the antibody from which the fragment was derived.

An "analog" is an amino acid or peptide sequence modified by replacement of at least one amino acid, modification or chemical substitution of an amino acid, which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity, of the unmodified sequence.

An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to degeneracies in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

An "engineered antibody" is a type of fusion protein, i.e., a synthetic antibody (e.g., a chimeric or humanized antibody) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody is replaced by analogous parts of CDRs from one or more donor antibodies which have specificity

for the selected epitope. These engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise immunoglobulin constant regions and variable framework regions from the acceptor antibody, and one or more CDRs from the *Plasmodium* donor antibodies described herein. Preferably the engineered antibodies of the invention will be produced by recombinant DNA technology.

"Chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region light chain and heavy chains (both CDR and framework regions) derived from a non-human donor mAb in association with light and heavy chain constant regions derived from a human (or other heterologous animal) acceptor mAb.

"Humanized antibody" refers to an engineered antibody having its CDRs and/or other portions of its light and/or heavy chain variable domain framework regions derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulins. Such antibodies can also include engineered antibodies characterized by a humanized heavy chain associated with a donor or acceptor unmodified light chain or a chimeric light chain, or vice versa.

"Effector agents" refers to non-protein carrier molecules to which the fusion proteins, and/or natural or synthetic light or heavy chain of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, or other

non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin. Such effector agents are useful to increase the half-life of the anti-*Plasmodium* derived amino acid sequences or to add to its properties.

II. *Anti-Plasmodium Antibodies*

For use in constructing the recombinant antibodies of this invention as it relates to malaria-causing pathogens, non-human species may be employed to generate a desirable donor antibody upon presentment with an antigen from a *Plasmodium* strain capable of infecting humans. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to the selected antigen. As one example, the murine mAb, NFS2, has been identified as a desirable antibody which may be employed for use in developing a chimeric or humanized antibody of this invention.

Murine IgG mAb NFS2 is characterized by an antigen binding specificity to the repeat region of the *P. falciparum* CS protein. In *in vitro* assays, it prevented invasion of sporozoites into human hepatocytes or hepatoma cells. Analogous antibodies in the mouse model have conferred passive protection against malaria. The production of mAb NFS2 is described in detail in Example 1 below.

This invention is not limited to the use of the illustrative NFS2 mAb or its hypervariable sequences. Wherever in the following description the donor mAb is identified as NFS2, this designation is made for simplicity of description only. Other anti-*Plasmodium* antibodies may be substituted therefor. Suitable antibodies include, for example, the murine mAb 2A10, which is directed against the CS repeat protein or other

mAbs described in R. A. Wirtz et al, Bull WHO, 65:39-45 (1987).

Antibodies produced in other animals protected by immunization with sporozoites or a protective epitope of a selected *Plasmodium* species may be similarly employed in this invention as a source of protective anti-*Plasmodium* sequences.

For example, the *P. falciparum* CS protein repeat region protein R32tet32 NH_2-Met-Asp-Pro-[(Asn-Ala-Asn-Pro)$_{15}$(Asn-Val-Asp-Pro)$_1$]$_2$-Leu-Arg-Arg-Thr-His-Arg-Gly-Arg-His-His-Arg-Arg-His-Arg-Cys-Gly-Cys-Trp-Arg-Leu-Tyr-Arg-Arg-His-His-Arg-Trp-Gly-Arg-Ser-Gly-Ser-COOH SEQ ID NO: 2 may be employed to elicit both human and murine mAbs with binding specificity therefor. This repeat region protein is a suitable target for screening for neutralizing antibodies useful in prophylactic agents against malarial infection.

Similarly, the epitope to which NFS2 is responsive, and analogs thereof, may be useful in the screening and development of additional *P. falciparum* antibodies, for use in the development of prophylactic compositions for short-term protection of humans against malaria. Other epitopes of interest include non-repetitive flanking region epitopes, other repeat domains or various liver, and blood and sexual stage epitopes of *Plasmodium* species. Knowledge of these epitopes enables one of skill in the art to define synthetic, and to identify naturally-occurring, peptides which would be suitable to confer passive or active immunity against *P. falciparum* or other *Plasmodium* species. This knowledge also permits the production of mAbs useful in the prophylaxis of malarial infection in humans.

For example, other *P. falciparum* antibodies may be developed by screening hybridomas or other combinatorial libraries, or antibody phage displays [W.

D. Huse et al., Science, 246:1275-1281 (1988)] using the murine mAb epitope described herein. A collection of antibodies, including hybridoma products or antibodies derived from any species immunoglobulin repertoire may be screened in a conventional competition assay, such as described in the examples below, with one or more epitopes described herein.

Antibodies such as those described above, including those generated against a desired epitope and produced by conventional techniques, including without limitation, genes encoding murine mAbs, human mAbs, and combinatorial antibodies, may be useful as donor antibodies, as sources of antibody fragments, as well as in prophylactic compositions against *P. falciparum* in humans. Preferably, the antibodies developed in response to *Plasmodium*, particularly *P. falciparum*, epitopes may be useful as donors of desirable variable heavy and/or light chain amino acid sequences, or functional fragments thereof (e.g., CDRs) useful in the development of fusion proteins, including engineered antibodies. Thus, the invention may utilize a donor antibody, other than NFS2, which is capable of binding to the *P. falciparum* peptide consisting essentially of the amino acid sequence of the repeat protein and analogs thereof.

Additionally, the mAbs identified herein, other mAbs which are developed and are responsive to the use of the sporozoites, R32tet32 [SEQ ID NO: 2] or the repeat epitopes identified herein may be further altered or manipulated to impart additional desirable prophylactic characteristics.

III. *Antibody Fragments, Amino Acid and Nucleotide Sequences*

The present invention provides isolated naturally-occurring or synthetic variable light chain and variable heavy chain sequences derived from mAb NFS2, as

well as CDRs and fragments therefrom, which may be employed in the design of fusion proteins (including engineered antibodies) which are characterized by the antigen binding specificity of this mAb.

The naturally-occurring variable heavy chain of NFS2 is characterized by the amino acid and encoding nucleic acid sequences illustrated in Fig. 4 [SEQ ID NOS: 9 and 10]. This chain is characterized by CDRs having the following nucleotide and predicted amino acid sequences. CDR 1 is characterized by the sequence:

AGCTATGCCATGTCT SEQ ID NO: 32
SerTyrAlaMetSer SEQ ID NO: 33.

The naturally occurring CDR 2 nucleic acid and amino acid sequences are SEQ ID NOS: 17 and 18, respectively:

GAAATTAGTGATGGTGGTAGTTACACCTACTATCCAGACACTGTGACGGGC
GluIleSerAspGlyGlySerTyrThrTyrTyrProAspThrValThrGly.

The naturally-occurring CDR 3 has the nucleic acid and amino acid sequences SEQ ID NOS: 19 and 20, respectively:

CTCATCTACTATGGTTACGACGGGTATGCTATGGACTAC
LeuIleTyrTyrGlyTyrAspGlyTyrAlaMetAspTyr.

Synthetic humanized variable heavy chains of NFS2 are characterized by the amino acid and encoding nucleic acid sequences illustrated in Fig. 5 [SEQ ID NOS: 9 and 10] and Fig. 6 [SEQ ID NOS: 13 and 14]. In both synthetic chains, the CDRs have the following nucleotide and predicted amino acid sequences. Nucleotide changes were made in CDR 1 from the naturally occurring CDRs, and are indicated by underlining. Synthetic CDR 1 is characterized by the sequence:

AGCTATGCCATG<u>AGC</u> SEQ ID NO: 15
SerTyrAlaMetSer SEQ ID NO: 16.

The synthetic CDR 2 nucleic acid and amino acid sequences are identical to the naturally-occurring sequences SEQ ID NOS: 17 and 18, respectively. The synthetic CDR 3 has the same nucleic acid and amino acid sequences as does

the naturally occurring CDR 3 SEQ ID NOS: 19 and 20, respectively.

The naturally occurring variable light chain of NFS2 is characterized by the amino acid sequence and encoding nucleic acid sequence of Fig. 1 [SEQ ID NOS: 3 and 4]. This chain is further characterized by CDRs having the following nucleotide and amino acid sequences. CDR 1 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 34 and 35, respectively:

AAGTCCAGTCAGAGCCTTTTATATAGTAGCAATCAAAAGAATTACTTGGCC
LysSerSerGlnSerLeuLeuTyrSerSerAsnGlnLysAsnTyrLeuAla.

CDR 2 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 36 and 37, respectively:

TGGGCATCCACTAGGGAATCT
TrpAlaSerThrArgGluSer.

CDR 3 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 38 and 39, respectively:

CAGCAATATTATAGCTATCCTCGGACG
GlnGlnTyrTyrSerTyrProArgThr.

A synthetic humanized variable light chain of NFS2 is characterized by the amino acid and encoding nucleic acid sequences illustrated in Fig. 2 [SEQ ID NOS: 5 and 6]. This chain is characterized by CDRs having the following predicted amino acid sequences and encoded by the illustrated nucleotide sequences. Nucleotide changes were made in the three CDRs from the naturally occurring corresponding CDRs, and are indicated by underlining. Synthetic CDR 1 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 21 and 22, respectively:

AAGAGCTCTCAGAGCCTTTTATACTCGAGCAATCAAAAGAATTACTTGGCC
LysSerSerGlnSerLeuLeuTyrSerSerAsnGlnLysAsnTyrLeuAla.

Synthetic CDR 2 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 23 and 24, respectively:

TGGGCGTCAACTAGGGAATCT
TrpAlaSerThrArgGluSer.

Synthetic CDR 3 is characterized by the amino acid sequences SEQ ID NO: 26 and encoding nucleotide sequences SEQ ID NO:25, respectively:

CAGCAATATTATAGCTATCCGCGGACG
GlnGlnTyrTyrSerTyrProArgThr.

Another synthetic humanized variable light chain of NFS2 is characterized by the amino acid and encoding nucleic acid sequences illustrated in Fig. 3 [SEQ ID NOS: 7 and 8]. This chain is characterized by identical CDRs 1 and 3 as in the Fig. 2 synthetic sequences. However, a nucleotide change was made in the CDR 2 from both the naturally occurring corresponding CDR 2, and the Fig. 2 synthetic CDR 2. Double underlining is employed to indicate the change from the Fig. 2 synthetic sequences. Synthetic CDR 2 is characterized by the nucleic acid and amino acid sequences SEQ ID NOS: 40 and 41, respectively: TGGGCGTCGACTAGGGAATCT
TrpAlaSerThrArgGluSer.

The present invention also includes the use of F_{ab} fragments or $F_{(ab')2}$ fragments. A F_{ab} fragment contains the entire light chain and amino terminal portion of the heavy chain; and a $F_{(ab')2}$ fragment is the fragment formed by two F_{ab} fragments bound by disulfide bonds. MAb NFS2 and engineered antibodies derived therefrom and described below provide sources of F_{ab} fragments and $F_{(ab')2}$ fragments which can be obtained by conventional means, e.g. cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. The F_{ab} fragments or $F_{(ab')2}$ fragments may be derived from any of the mAbs described above, as agents protective *in vivo* against infection by malarial pathogens, particularly *P. falciparum*.

The variable chain peptide sequences of murine mAb NFS2, its variable chain peptide sequences and CDRs, functional fragments, F_{ab} fragments, and analogs thereof, and the nucleic acid sequences encoding them, may be

useful in obtaining various fusion molecules encoding desired fusion proteins, particularly engineered antibodies, and in methods for preparing and administering pharmaceutical compositions containing them.

The nucleic acid sequences of the invention, or fragments thereof, encoding the variable light chains and heavy chain peptide sequences or CDR peptides, or functional fragments thereof are used in unmodified form or are synthesized to introduce desirable modifications. The isolated naturally-occurring or synthetic nucleic acid sequences, which are derived from mAB NFS2 or from other desired anti-*Plasmodium* antibodies, may optionally contain restriction sites to facilitate insertion or ligation into a suitable nucleic acid sequence encoding a desired antibody framework region, ligation with mutagenized CDRs, or fusion with a nucleic acid sequence encoding a selected second fusion partner.

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention, e.g., Figs. 1 - 6 [SEQ ID NOS: 3-26], and functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated or synthetic nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs or functional fragments thereof can be used to produce fusion proteins, i.e. chimeric or humanized antibodies, or other engineered antibodies of this invention, when operatively combined with a second fusion partner.

These sequences are also useful for mutagenic insertion of specific changes within the nucleic acid sequences encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic

acid sequence into a vector for expression. For example, silent nucleotide substitutions may be made in the nucleotide sequences encoding the CDRs to create restriction enzyme sites to facilitate insertion of the mutagenic frameworks, or to modify the selected frameworks at nucleotide positions analogous to those of the donor antibody. Such mutations may include those inserted for the purpose of contributing to higher antigen binding affinity.

IV. *Fusion Molecules and Fusion Proteins*

Fusion molecules of this invention can encode engineered antibodies, chimeric antibodies and humanized antibodies. A desired fusion molecule may contain a first fusion partner encoding an amino acid sequence having the antigen specificity of a *Plasmodium* antibody directed against the amino acid sequence of the repeat protein and analogs thereof, operatively linked to a second fusion partner. Desirably the source of the first fusion partner is a selected mAb, e.,g., mAb NFS2, the source of nucleic acid sequences of Figs. 1 [SEQ ID NO: 3] and 4 [SEQ ID NO: 9].

A fusion molecule may encode an amino acid sequence for a naturally occurring variable heavy chain sequence of Fig. 4 [SEQ ID NOS: 9 and 10], a functional fragment or analog thereof, a naturally occurring variable light chain sequence of Fig. 1 [SEQ ID NO: 3 and 4], a functional fragment or analog thereof, or one or more NFS2 CDRs [SEQ ID NO: 15-26]. Another exemplary fusion molecule may encode a synthetic variable heavy and/or light chain from the donor mAb, such as those of Figs. 2 [SEQ ID NOS: 5 and 6], 3 [SEQ ID NOS: 7 and 8], 5 [SEQ ID NOS: 11 and 12], and 6 [SEQ ID NOS: 13 and 14], having the antigen specificity of *P. falciparum* antibody.

A desirable fusion molecule of this invention may be characterized by encoding an amino acid sequence

containing at least one, and preferably all of the CDRs [SEQ ID NOS: 15-26] of the variable region of the heavy and/or light chains of the murine antibody NFS2, or a functional fragment or analog thereof.

The second fusion partners are defined above, and may include a sequence encoding a peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of NFS2. One example is a sequence encoding a second antibody region of interest and may optionally include a linker sequence.

The resulting fusion molecule may encode both anti-*P. falciparum* antigen specificity and the characteristic of the second fusion partner, e.g., a functional characteristic such as secretion from a recombinant host, or a therapeutic characteristic if the fusion partner itself encodes a therapeutic protein, or additional antigenic characteristics, if the fusion partner encodes a protein having its own antigen specificity.

If the second fusion partner is derived from another antibody, e.g., any isotype or class of immunoglobulin framework or constant region (preferably human), or the like, an engineered antibody is provided. Thus, for example, a fusion molecule of this invention may comprise a complete antibody molecule, having full length heavy and light chains (Figs. 4 [SEQ ID NOS: 9 and 10] and 1 [SEQ ID NOS: 3 and 4]). For example, the invention includes isolated naturally-occurring or synthetic nucleic acid sequences, which may encode variable region sequences, CDR peptides, fragments thereof derived from desired *Plasmodium* mAbs, any fragment of an engineered antibody, such as the F_{ab} or $F_{(ab')2}$ fragment, a heavy chain dimer, or any minimal recombinant fragment thereof such as an F_v or a single-chain antibody (SCA) or any other sequence encoding a

protein with the same specificity as the selected mAb, e.g., the *Plasmodium* mAb NFS2.

The first fusion partner may also be associated with effector agents as defined above, to which the first fusion partner may be operatively linked by conventional means, e.g., attached to the NFS2 encoding nucleic acids by a covalent bridging structure.

Fusion or linkage between the first fusion partners and the selected second fusion partner may be by way of any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or heterobifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Where the first fusion partner is associated with an effector agent, non-proteinaceous, conventional chemical linking agents may be used to fuse or join the anti-*P. falciparum* amino acid sequences to the effector agent. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional inert linker sequences which simply provide for a desired amount of space between the fusion partners or between the first fusion partner and the effector agent may also be constructed into the fusion molecule. The design of such linkers is well known to those of skill in the art.

Expression of such fusion molecules results in fusion proteins of this invention. One particularly desirable type of fusion protein includes the engineered antibody in which, at a minimum, fragments of the variable heavy and/or light domains of an acceptor mAb have been replaced by analogous parts of the variable light and/or heavy chains from one or more donor monoclonal antibodies, which include the *Plasmodium* mAbs described herein, such as NFS2.

One example of a particularly desirable engineered antibody is a humanized antibody, in which CDRs from a desired donor murine mAb are inserted into the framework regions of a human antibody. A preferred donor antibody is one directed against a *Plasmodium* epitope, preferably one specific for the repeat region epitope of *P. falciparum*. A particularly preferred donor antibody has all or a portion of the variable domain amino acid sequences of NFS2. In these humanized antibodies one, two or preferably three CDRs from the *Plasmodium* antibody heavy chain and/or light chain variable regions are inserted into the framework regions of a selected human antibody, replacing the native CDRs of that latter antibody.

Preferably, the variable domains in both human heavy and light chains have been altered by CDR replacement. This engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof. Such humanized antibodies may or may not also include minimal alteration of the acceptor mAb light and/or heavy variable domain framework region in order to retain donor mAb binding specificity. The humanized antibody possesses the combination of properties required for effective prevention and treatment of infectious *P. falciparum* disease in animals or man.

The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin. A suitable human antibody may be one selected from a conventional database, e.g., the Kabat database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy

chain constant region and/or a heavy chain variable framework region for the insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same human antibody.

Desirably, the heterologous framework and constant regions are selected from the human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

As one example, an engineered antibody may be encoded by a synthetic nucleic acid sequence encoding CDRs of the variable light chain region of NFS2 or a functional fragment thereof in place of at least a part of the nucleic acid sequence encoding the light chain variable region of an acceptor mAb, and a nucleic acid sequence encoding CDRs of the variable heavy chain region of NFS2 or a functional fragment thereof in place of at least a part of the nucleic acid sequence encoding the heavy chain variable region of an acceptor mAb, such as a human antibody. The resulting humanized antibody is characterized by the antigen binding specificity of mAb NFS2.

Alternatively, the engineered antibody (or the other monoclonal antibodies) of the invention may have attached to it an effector or reporter molecule. Alternatively, the procedure of recombinant DNA technology may be used to produce an engineered antibody

of the invention in which the F_c fragment or CH3 domain of a complete antibody molecule has been replaced by an enzyme or toxin molecule.

It will be understood by those skilled in the art that such an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity of the donor antibody. It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both. Such engineered antibodies can be effective in prevention of productive malaria (e.g., by *P. falciparum*) infection in humans.

Additionally, the invention provides fusion proteins which are chimeric antibodies, as defined above. Such antibodies differ from the humanized antibodies described above by providing the entire donor antibody heavy chain and light chain variable regions, including framework regions, e.g., Figs. 1 [SEQ ID NOS: 3 and 4] and 4 [SEQ ID NOS: 9 and 10], fused to acceptor constant regions for both chains.

V. *Production of Proteins and Antibodies*

A fusion molecule, recombinant antibody or fusion protein of this invention is desirably constructed by recombinant DNA technology using genetic engineering techniques. The same or similar techniques may also be employed to generate other embodiments of this invention, e.g., to construct the chimeric or humanized antibodies, the synthetic light and heavy chains, the CDRs, and the nucleic acid sequences encoding them, as above mentioned.

A specific embodiment of the compositions of this invention is set out in Example 3 below using the CDRs of murine NFS2 and one or more selected human antibody light and heavy chain framework regions. Briefly described, a hybridoma producing the murine

antibody NFS2 is conventionally cloned, and the cDNA of its heavy and light chain variable regions is obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook et al., Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989). The variable regions of the NFS2 are obtained using PCR primers, and the CDRs are identified using a known computer database, e.g, Kabat, for comparison to other antibodies.

Homologous framework regions of a heavy chain variable region from a human antibody were identified using the same databases, e.g., Kabat, and a human antibody having homology to NFS2 was selected as the acceptor antibody. The sequences of synthetic heavy chain variable regions containing the NFS2 CDRs within the human antibody frameworks were designed with optional nucleotide replacements in the framework regions for restriction sites. This designed sequence was synthesized by overlapping oligonucleotides, amplified by polymerase chain reaction (PCR), and corrected for errors.

A suitable light chain variable framework region was designed in a similar manner, resulting in two synthetic light chain variable sequences containing the NFS2 CDRs. See, Figs. 2 [SEQ ID NOS: 5 and 6] and 3 [SEQ ID NOS: 7 and 8]. As stated above, the source of the light chain is not a limiting factor of this invention.

These synthetic variable light and/or heavy chain sequences and the CDRs of mAb NFS2, and their encoding nucleic acid sequences, are employed in the construction of fusion proteins and engineered antibodies, preferably humanized antibodies, of this invention, by the following process. By conventional techniques, a DNA sequence is obtained which encodes the donor antibody variable heavy or light chain regions,

wherein at least the CDRs and those minimal portions of the acceptor mAb light and/or heavy variable domain framework region required in order to retain donor mAb binding specificity as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin.

A conventional expression vector or recombinant plasmid is produced by placing these sequences encoding the fusion protein in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Such regulatory sequences may be readily selected by one of skill in the art and are not intended as a limitation of the present invention. Regulatory sequence include promoter sequences, e.g., CMV promoter, and signal sequences which can be derived by one of skill in the art from antibodies.

A first vector can contain a sequence encoding a light chain-derived polypeptide. Similarly, a second expression vector is produced having a similar DNA sequence which encodes a complementary antibody light or heavy chain. Preferably at least the CDRs (and those minimal portions of the acceptor mAb light and/or heavy variable domain framework region required in order to retain donor mAb binding specificity) of the variable domain are derived from a donor antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin in these vectors. Preferably this second expression vector is identical to the first, with the exception of the coding sequences and selectable markers, to ensure that each polypeptide chain is functionally expressed.

In another alternative, a single vector of the invention may be used, the vector including the sequence

encoding both light chain and heavy chain-derived polypeptides. The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or the single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as an ELISA assay followed by the Inhibition of Sporozoite Invasion (ISI) assay described in the examples below. Similar conventional techniques may be employed to construct other fusion proteins of this invention.

Thus, the invention also includes a recombinant plasmid containing the coding sequence of the fusion molecule or engineered antibody of the invention. Such a vector is prepared by conventional techniques and suitably comprises the above-described DNA sequences and a suitable promoter operatively linked to the DNA sequences which encode the engineered antibody. Such a vector is transfected into a mammalian cell via conventional techniques.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United

Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and marker genes, and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences which are in operative association with the DNA coding sequences of the immunoglobulin regions and capable of directing the replication and expression of heterologous DNA sequences in selected host cells, such as CMV promoters. These vectors contain the above described DNA sequences which code for the engineered antibody or other fusion protein. Alternatively, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by marker genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR) or neomycin resistance gene (neo^R). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH), and the betaglobin promoter sequence (betaglupro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures for use in directing the expression of the recombinant DNA in a selected host. Other appropriate

expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

Two exemplary expression vectors employed in the following examples for expression of the synthetic heavy and light chain sequences are the mammalian vectors Pfhzhc2-3-Pcd and Pfhzlc1-1-Pcn (see Figs. 7 and 8). However, this invention is not limited to the use of these illustrative pUC19-based vectors.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or other fusion protein described by this invention. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of the mAbs of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or other protein of the invention of this invention are preferably a eukaryotic cell, and most preferably a mammalian cell, such as a CHO cell or a myeloid cell. Other primate cells may be used as host cells and, most desirably, human cells are used, thus enabling the protein to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant mAbs of the present invention. However, due to the tendency of

proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant mAb produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the protein expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells and viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the fusion protein, and preferably an engineered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the fusion proteins, preferably the engineered antibodies of the invention, may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

The engineered antibody is then examined for *in vitro* activity by use of an assay appropriate for the selected pathogen. Presently conventional ELISA assay

formats are employed to assess qualitative and quantitative binding of the engineered antibody to the R32tet32 epitope [SEQ ID NO: 2]. The ISI assay described in Example 6 may also be employed. A similar assay, the inhibition of hepatocyte invasion assay (ILSDA), may be performed [S. Mellouk et al., Bull. WHO, Suppl. 68:52-58 (1990)]. Additionally, assays currently being developed in the SCID mouse model may also be used to verify efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

The examples below demonstrate the method for constructing the humanized antibodies derived from the murine mAb NFS2. Following the procedures described for humanized antibodies prepared from this antibody, one of skill in the art may also construct humanized antibodies from other malarial antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the altered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies can effectively passively protect a human against *P. falciparum* infection.

VI. *Therapeutic/Prophylactic Uses*

The fusion proteins, particularly the engineered antibodies described above, functional fragments, analogs and the other protein or peptides described herein may be employed as prophylactic agents, capable of conferring short-term passive immunity to infection by the pathogen from which the original antigenic substance derives, e.g., *P. falciparum*, to a

subject. The protective effect conferred by the use of the engineered antibodies of this invention is produced by binding of the immunoglobulin to the pathogen and the subsequent removal of this bound complex by the normal function of macrophages. Thus, the engineered antibodies of the present invention, when in preparations and formulations appropriate for prophylactic use, are highly desirable for persons anticipating short-term exposure to the pathogen, e.g., travelers, tourists, or military personnel anticipating travel in endemic areas.

Therefore, this invention also relates to a method of prophylactic treatment of human *P. falciparum* infection in a human in need thereof which comprises administering an effective, protective dose of antibodies including one or more of the engineered antibodies or other fusion proteins described herein, or fragments thereof, to a human anticipating exposure to a species of *Plasmodium*.

The fusion proteins, including the engineered antibodies or fragments thereof of this invention, may also be used in conjunction with other antibodies, particularly human mAbs reactive with other epitopes responsible for the disease against which the engineered antibody of the invention is directed. Similarly mAbs reactive with other epitopes responsible for the disease in a selected animal against which the antibody of the invention is directed may also be employed in veterinary compositions. Any antibody that is capable of operating without interfering with the *Plasmodium* antibody of this invention, e.g., antibodies to other malaria stages or to different epitopes, are useful in these compositions.

The prophylactic agents of this invention are believed to be desirable to confer protection to exposure to the pathogen for from about 4 days to about 8 weeks, without requiring booster dosages of the agent. This

definition of 'short-term' relates to the relative duration of the recombinant antibodies of the present invention in the human circulation.

The mode of administration of the prophylactic agent of the invention may be any suitable route which delivers the agent to the host. The fusion proteins, including the engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. However, the agent is preferably administered by intramuscular injection.

Prophylactic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered antibody of the invention as an active ingredient in a nontoxic and sterile pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., saline, glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can

vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for parenteral, e.g., intramuscular injection, could be prepared to contain 1 mL sterile buffered water, and between about 50 to about 100 mg of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the prophylactic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively prevent *P. falciparum* infection in a human or other animal, one dose of approximately 1 mg/kg to approximately 20 mg/kg of a protein or an antibody of this invention should be administered parenterally, preferably intramuscularly (i.m.) and possibly intravenously (i.v.). Such dose may be repeated at appropriate intervals during exposure.

The antibodies, engineered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with

conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dosage levels and pattern being selected by the treating physician. In any event, the pharmaceutical composition of the invention should provide a quantity of the engineered antibodies of the invention sufficient to effectively prevent infection.

The following examples illustrate the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional codes or by full name, unless otherwise indicated. All restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning, ligation and other recombinant DNA methodology were as performed in Sambrook et al., cited previously, or the first edition thereof.

Example 1 - Description of Production of NFS2

Murine IgG mAb NFS2 was made by repeated injection of *P. falciparum* sporozoites into mice followed by B cell fusion with a myeloma cell line. The murine mAb NFS2 is characterized by an antigen binding specificity to the repeat region of the *P. falciparum* CS protein. Specifically, the NFS2 mAb binds to the epitope, Pro Asn Ala Asn Pro Asn SEQ ID NO: 27. It is possible that the antibody also binds to a larger, or overlapping epitope on the repeat region.

This murine mAb, in *in vitro* assays, prevented invasion of sporozoites into human hepatocytes and hepatoma cells. Analogous antibodies in the mouse model have conferred passive protection against malaria and have been observed to be highly potent [see, e.g., R. A. Wirtz et al., Bull WHO, 65:39-45 (1987), incorporated herein by reference]. This antibody is available from the U. S. Naval Medical Research Institute.

Example 2 - Cloning and Sequencing of NFS2

Cytoplasmic RNA was prepared by the method of Favaloro et al., Meth. Enzymol., 65:718-749 (1980) from NFS2, and hybridoma cell lines. The following primers were used in the synthesis of Ig heavy (V_H) and light (V_L) chain variable region cDNAs, respectively. The V_L primers, #2580 and #2789, extended from HindII through XbaI and were made to conserved regions of murine RNA.

HindIII

#2580: 5'CCAGATGTAAGCTTCAGCTGACCCAGTCTCCA3' SEQ ID NO: 28
PvuII

Xba I NaeI

#2789:
5'CATCTAGATGGCGCCGCCACAGTACGTTTGATCTCCAGCTTGGTCCC3' SEQ ID NO: 29 The V_H primers, #2621 and #2853, extended from KpnI through PstI and were made to conserved regions of murine RNA.

KpnI XhoI

#2621: 5'GGGGTACCAGGTCCA(A/G)CT(G/T)CTCGAGTC(A/T)GG3'
SEQ ID NO: 30

PstI

#2853: 5'GCCTGCAGCTAGCTGAGGAGACGGTGACCGTGGTCCCTTGG-
NheI

CCCCAG3' SEQ ID NO: 31

PCR, as described by Saiki et al., Science, 239:487-491 (1988), was performed on the RNA template. For the PCR, the primers used were identified above. For

PCR amplification of V_H, DNA/primer mixtures consisted of 5 μl RNA and 0.5 μM of the primers. For PCR amplifications of V_L, DNA/primer mixtures consisted of 5 μl RNA and 0.5 μM of the primers. To these mixtures was added 250 μM each of dATP, dCTP, dGTP and dTTP, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.01% (v/v) Tween 20, 0.01% (v/v) Nonidet P40 and 5 units AmpliTaq [Cetus]. Samples were subjected to 25 thermal cycles of PCR at 94°C, 30 seconds; 55°C, 30 seconds; 72°C, 45 seconds; ending with 5 minutes at 72°C. For cloning and sequencing, amplified V_H DNA was purified on a low melting point agarose gel and by Elutip-d column chromatography [Schleicher and Schuell-Dussel, Germany] and cloned into pUC18 [New England Biolabs]. The general cloning and ligation methodology was as described in Maniatis et al., cited above.

V_H DNA was cloned as KpnI-PstI fragments into similarly-digested pUC18. V_L DNA was cloned as HindIII-XbaI fragments into pUC18 digested with the same enzymes. Representative clones were sequenced by the dideoxy method [Sanger et al., Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977)] using T7 DNA polymerase [US Biologicals]. From the sequences of NFS2 V_H and V_L domains, the CDR sequences were elucidated in accordance with the methodology of Kabat et al., in "Sequences of Proteins of Immunological Interest", US Dept of Health and Human Services, US Government Printing Office (1987) utilizing computer assisted alignment with other V_H and V_L sequences. The CDRs of the heavy and light chains of NFS2 are listed above and identified herein as SEQ ID NOS: 15-20 and 21-26.

Example 3 - Humanized Antibodies

The following example describes the preparation of an exemplary engineered antibody. Similar procedures may be followed for the development of engineered antibodies, using other *Plasmodium* antibodies or other anti-pathogen antibodies developed by conventional means.

The source of the donor CDRs utilized to prepare these engineered antibodies was the murine mAb, NFS2, described in Examples 1 and 2 above. The sequenced NFS2 variable framework regions were employed to again search through the Kabat database to identify homologous framework regions of a human antibody. The framework region of an antibody obtained from a human SLE patient B-cell hybridoma cell line 18/17 [H. Dersimonian et al., J. Immunol., 139:2496-2501 (1987)] was determined to be approximately 80% homologous to the NFS2 variable heavy chain framework region.

Given the murine NFS2 CDRs (Example 2) and the sequence of the human antibody 18/17, a synthetic heavy chain variable region was made, and PCR performed to fill in and amplify DNA. The NFS2 CDR sequences and the 18/17 V_H framework regions were synthesized by the following overlapping oligonucleotides:

SEQ ID NO:44:TAGTGAAGAATTCGAGGACGCCAGCAACATGGTGTTGCAGAC
CCAGGTCTTCATTTCTCTGTTGCTCTGGATCTCTGGTGCCTACGGGGAGGTGCAG
(Base 1-97);

SEQ ID NO:45:GCTAGCGGATTCACCTTTAGCAGCCATGTCGGACCCCCCAGG
GACTCTGAGAGGACACGTCGATCGCCTAAGTGGAAATCCTATGCCATGAGCTGGG
TCCGCCAGGCTCCAGGGAAAGGTCTAGAGTGGGTCTCAGAAATCTTTATAGTGAT
GGTGGTAGTTAC (Base 158-259);

SEQ ID NO:46: GAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAG
GACACGTCTCTGTTAAGGTTCTTGTGCGACATAGACGTTTACTGCAGTATATTAC
TGTGCGAAACTCATCTACTATGGTTACGACGGGTATGCTATGGACTAGCTGCCCA
TACGATACCTGATC (Base 316-421);

SEQ ID NO:47: TTCTTGAAAGCTTGGGCCCTTGGTACTAGCTGAGCTCACGG

TGACCAGGGTACCCTGGCCCCAGTAGTCCATAGCATACCCGTCG (Base 484-400);
SEQ ID NO:48: CATTTGCAGATACAGCGTGTTCTTGGAATTGTCTCTGGATA TCGTGAACCGGCCCGTCACAGTGTCTGGATAGTAGGTGTAACTACCACCATCACT AATTTC (Base 337-236);
SEQ ID NO:49: CTAAAGGTGAATCCGCTAGCTGCACAGGAGAGTCTCAGGGA CCCCCCAGGCTGTACCAAGCCTCCCCCAGACTCGAGCAGCTGCACCTCCCCGTAG GCACC (Base 177-77).

These primers are annealed together and DNA is filled in using Taq polymerase, followed by PCR amplification using the following 5' primer:
SEQ ID NO:50: CCGCGAATTCGAGGACGCCAGCAAC
and 3' primer: SEQ ID NO:51:
CCGCAAGCTTGGGCCCTTGGTACTAGCT.

Any errors in the mapped sequence which were inserted by PCR were corrected. In addition, conservative nucleotide replacements were placed in the framework regions to introduce selected restriction sites suitable for enzymatic cleavage. These alterations in the framework regions are indicated by boxes in the sequences of Figs. 2 [SEQ ID NOS: 5 and 6], 3 [SEQ ID NOS: 7 and 8], 5 [SEQ ID NOS: 11 and 12] and 6 [SEQ ID NOS: 13 and 14]. Additionally, most murine and human antibodies have a basic residue before CDR3. Because the variable heavy chain of NFS2 has a non-basic residue Ser before CDR3 [SEQ ID NOS: 19-20 and 25 and 26], the acceptor basic residue (Lys) before CDR3 was deleted and replaced with Ser to create heavy chain Pfhzhc2-3.

Two synthetic heavy chain variable regions were obtained, namely, Pfhzhc2-3 SEQ ID NOS: 13 and 14, and Pfhzhc2-4 SEQ ID NOS: 11 and 12. These sequences are described in detail in Figs. 5 and 6. Each of these synthetic heavy chain variable regions is characterized by one or two nucleotide, or amino acid, differences. For example, Pfhzhc2-3 [SEQ ID NOS: 13 and 14] has a Ser

at position 98; and Pfhzhc2-4 [SEQ ID NOS: 11 and 12] has a Lys at position 98. Otherwise, these heavy chain variable regions are identical.

For a suitable light chain variable framework region, the NFS2 light chain CDRs and the light chain variable framework sequence of the human antibody identified in H. G. Klobeck et al., Nucl. Acids Res., 13:6515-6529 (1985) were used to make a suitable synthetic light chain sequence by the same methods. The oligonucleotides used were as follows.

SEQ ID NO:52: TAAGCGGAATTCGTAGTCGGATATCGTGATGACCCAGTC TCCAGACTCGCTAGCTGTGTCTCTGGGCGAGAGGGC (Base 1-75);

SEQ ID NO:53: TTACTTGGCCTGGTATCAGCAGAAACCCGGGCAGTCTCC TAAGTTGCTCATAGTTTTCTTAATGAACCGGACTTACTGGGCGTCAACTAG (Base 130-198);

SEQ ID NO:54: GACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAA GATGTGGCAGTATACTACTGCTGTCTAAAGTGTCAGCAATATTATAGCTATCC (Base 241-321);

SEQ ID NO:55: CAGTTGGAAAGCTTGGCGCCGCCACAGTACGTTTGATCTCCA CCTTGGTCCCTCCGCCGAACGTCCGCGGATAGCTATAATATTGC (Base 389-304);

SEQ ID NO:56: GTGAAATCTGTCCCAGACCCGCTGCCACTGAATCGG TCAGGTACCCCAGATTCCCTAGTTGACGCC (Base 252-187);

SEQ ID NO:57: CAGGCCAAGTAATTCTTTTGATTGCTCGAGTATAAA AGGCTCTGAGAGCTCTTGCAGTTGATGGTGGCCCTCTCGCCC (Base 141-64).

As described above, the primers were annealed together and DNA filled in using Taq polymerase, followed by PCR amplifcation with the following 5' SEQ ID NO:58: GCGGAATTCGTAGTCGGATATCGTGATGAC and 3' SEQ ID NO:59: TGGAAAGCTTGGCGCCGCCACAGTACGTTTGATC primers.

Two synthetic light chain variable sequences containing the NFS2 CDRs were designed and synthesized as described above for the synthetic heavy chains and referred to as Pfhzlc1-1 SEQ ID NOS: 5 and 6, and

Pfhzlc1-2 SEQ ID NOS: 7 and 8. These two sequences differed in amino acid sequence at only one amino acid position, 49. Pfhzlc1-1 [SEQ ID NOS: 5 and 6] has a Ser at position 49; Pfhzlc1-2 [SEQ ID NOS: 7 and 8] has a Pro at the same position.

These synthetic variable light and/or heavy chain sequences are employed in the construction of an exemplary humanized antibody. It is expected that any of the synthetic heavy chains will successfully associate with any of the synthetic light chains to produce a useful humanized antibody.

To produce a humanized antibody, for the heavy chain variable sequence Pfhzhc2-3 [SEQ ID NO: 13 and 14] (Fig. 6), the following signal sequence was synthesized onto this variable region: SEQ ID NOS: 60: ATGGTGTTGCAG ACCCAGGTCTTCATTTCTCTGTTGCTCTGGATCTCTGGTGCCTAC, which encodes SEQ ID NO:61: MetValLeuGlnThrGlnValPheIleSerLeu LeuLeuTrpIleSerGlyAlaTyr. For the synthetic light chain variable sequence Pfhzlc1-1 [SEQ ID NO: 5 and 6] (Fig. 2), the construct is digested with EcoRI and EcoRV, and the same signal sequence was ligated onto the variable sequence. Other signal sequences are well known to those of skill in the art and may be substituted for this exemplary sequence.

Selected constant regions of the human IgG_1 antibodies selected for the heavy and light chain were synthesized and confirmed by PCR. These constant region sequences were then inserted into pUC19-based expression vectors. The above-described synthetic variable constructs, containing the signal and variable regions of the light and heavy chains, were thereafter inserted into these pUC19-based expression vectors containing CMV promoters and the constant regions and fused in frame to the previously inserted human heavy and light chain

constant regions by conventional methods [Maniatis et al., cited above]. Thus, after insertion of the synthetic variable regions into these expression vectors, the plasmids shown in Figs. 7 and 8, resulted. These plasmids were then co-transfected into a selected host cell and, following incubation, the media was assayed for antibody activity via ELISA as described in Example 4 below.

Using similar techniques, another exemplary humanized antibody is constructed using the synthesized heavy chain sequence Pfhzhc2-3 [SEQ ID NO:13 and 14] (Fig. 6) and the synthetic light chain sequence Pfhzlc1-2 [SEQ ID NO:7 and 8].

Example 4 - A High Affinity Humanized Antibody

The amino acid differences in the variable regions of the frameworks of the original murine antibody NSF2 described in Examples 1 and 2 and the Pfhzhc2.3 were determined, and several changes were made to increase the level of conservation of the original antibody conformation.

At amino acid position 49, the Ser of the humanized heavy chain Pfhzhc2.3 was changed to Ala, which is the amino acid found at this position in the native murine NSF2. The replacement employed conventional genetic engineering technology, e.g., by making a synthetic DNA fragment containing the appropriate nucleotide changes to alter the amino acid. A fragment of Pfhzhc2.3 was digested with XbaI and EcoRV and the synthetic fragment bearing the nucleotide change encoding Ala in place of a Ser codon, is inserted to make the amino acid replacement. The resulting synthetic heavy chain was termed Pfhzhc2.6.

This synthetic heavy chain was expressed as previously described for the Pfhzhc2.3 synthetic heavy

chain. The expression plasmid for this humanized heavy chain sequence is essentially identical to the expression plasmid illustrated in Fig. 7, with the exception of the single amino acid difference described previously.

Similarly, humanized antibodies consisting of the Pfhzhc2.6 heavy chain and Pfhzlc1.1 light chain and the Pfhzhc2.6 heavy chain and the Pfhzlc1.2 light chain were assembled via co-transfection of mammalian cells and assayed for antibody activity by ELISA, as described in Example 5 below.

Other high affinity antibodies specific for *P. falciparum* can be developed using a similar method designed to achieve minimal variable region framework modifications. The method involves the following order of steps of alteration and testing:

(1) In addition to the alteration at amino acid position 49, other individual framework amino acid residues known to be critical for interaction with CDRs are compared in the primary antibody and the engineered CDR-replacement antibody. For example, heavy chain amino acid residue (Kabat numbering; see Kabat et al., cited above) is compared in the primary (donor) and engineered antibodies. A residue at this position is thought to interact with the invariant heavy chain CDR residue at position 94 (Lys-basic) via a salt bridge.

If an amino acid is in the framework of the donor antibody but not in the framework of the engineered antibody, then an alternative heavy chain gene comprising the engineered antibody is produced. In the reverse situation whereby the engineered antibody framework comprises a residue at one position but the donor antibody does not, then an alternative heavy chain gene comprising the original amino acid at that position is reproduced. Prior to any further analysis, alternative

plasmids produced on this basis are tested for production of high affinity engineered antibodies.

(2) Framework amino acids within four residues of the CDRs as defined according to Kabat (see Kabat et al., cited above) are compared in the primary antibody and engineered CDR-replacement antibody. Where differences are present, then for each region the specific amino acids of that region are substituted for those in the corresponding region of the engineered antibody to provide a small number of engineered genes. Alternative plasmids produced on this basis are then tested for production of high affinity antibodies.

(3) Framework residues in the primary and engineered CDR-replacement antibodies are compared and residues with major differences in charge, size or hydrophobicity are highlighted. Alternative plasmids are produced on this basis with the individual highlighted amino acids represented by the corresponding amino acids of the primary antibody and such alternative plasmids are tested for production of high affinity antibodies.

Example 5: ELISA Assays

Expression of the synthetic heavy chain and light chain sequences were tested by transiently transfecting the plasmid DNAs into monkey COS cells. The following results are reported for Pfhzhc2-3 and Pfhz1c1-1. Ten micrograms of the plasmids are mixed together and ethanol precipitated. The DNAs are dissolved in Tris buffered saline (TBS) and mixed with DEAE-dextran (400 μg/ml final concentration)/chloroquine (0.1 mm), added to 3-4 x 10^5 COS cells grown in T25 flask, incubated for 4 hours at 37°C. Cells were shocked with 10% DMSO in phosphate buffered saline (PBS) for 1-2 minutes and after washing with PBS, cells were incubated in the presence of serum free growth medium. Media were collected 72 hours

post transfection (day 3 sample) and fresh media were added which were collected 120 hours post transfection (referred to as day 5 sample).

To compare the binding affinity of various antibodies, i.e., chimeric and humanized, large scale COS transfections were performed as described above. For each antibody, 200 μg heavy chain plasmid and 200 μg light chain plasmid DNAs were used to transfect a total of 2.5×10^7 COS cells. The media collected (day 3 and day 5) were pooled, assayed for antibody expression using F_c capture ELISA. The media were concentrated using Amicon to 6 ml. Amount of antibody in the pooled media varied from 9 mg/ml to 25 mg/ml. These concentrated samples were used to compare binding affinities via the ISI and ILSDA assays.

The presence of humanized antibody in the medium of wells containing transfected clones is measured by conventional ELISA techniques. Micro-titer plates are coated overnight at 4°C with goat anti-human IgG (F_c specific) antibodies [Sigma, St. Louis, MO] at 0.1 μg per well. After washing with PBS (pH 7.5), 50 μl of culture medium from the wells containing transfectants is added to each microtitre well for 2 hours at room temperature. The wells are then emptied, washed with PBS and peroxidase-conjugated goat anti-human IgG antibodies [BioRad, Richmond, CA] are added at 50 μL of a 1/1000 dilution per well. Plates are then incubated at room temperature for 1 hour. The wells are then emptied and washed with PBS. 100 μl 2.2'-azino-di[3-ethyl-benzthiazoline sulfonate(6)] are added per well. Reactions were allowed to continue for 1 hour at room temperature. The absorbance at 405 nm is then measured spectrophotometrically. The ability of the humanized antibody in the medium of wells containing transfected clones to bind *P. falciparum* circumsporozoite protein was

measured by ELISA. Microtiter plates were coated overnight at 4°C with *E. coli*-produced R32tet32 at 0.1 μg per well. After washing with PBS, 50 μl of culture medium from the wells which contain transfectants is added to each microtiter well for 2 hours at room temperature. The wells are then emptied, washed with PBS and peroxidase-conjugated goat anti-human IgG antibodies [BioRad, Richmond, CA] are added at 50 μL of a 1/1000 dilution per well. Plates are then incubated at room temperature for 1 hour. The wells are then emptied and washed with PBS. 100 μl 2.2'-azino-di[3-ethyl-benzthiazoline sulfonate(6)] are added per well. Reactions were allowed to continue for 1 hour at room temperature. The absorbance at 405 nm is then measured spectrophotometrically.

In preliminary studies, an increase in affinity was observed for the humanized antibodies of Example 4, which contained the Pfhzhc2-6 heavy chain construct, as compared to the Pfhzhc2-3 heavy chain construct.

Example 6 - Construction of Chimeric Antibody

A chimeric antibody of the invention was constructed essentially as described above. A chimeric antibody contains the native murine NSF2 variable framework and CDR regions on the human constant regions selected for the heavy chain [H. Dersimonian et al., J. Immunol., 139:2496-2501 (1987) and light chain [Klobeck et al., Nucl. Acids. Res., 13:6515-6529 (1985)], with the exception that the variable regions were obtained by PCR of the RNA of the murine antibody obtained from the NFS2 hybridoma and the entire constant regions of the human IgG_1 antibodies were synthesized by overlapping oligonucleotides and amplified by PCR. Any errors which were inserted by PCR were corrected. The resulting

chimeric heavy chain and chimeric light chain were expressed as described above for the humanized antibody.

This chimeric antibody is advantageous in that it is characterized by activity substantially identical to that of the native murine antibody, but contains enough human sequences that it is anticipated to be useful in human therapy.

Example 7 - ISI Assay

The Inhibition of Sporozoite Invasion assay is performed as described in M. R. Hollingdale et al., J. Immunol., 132:909-913 (1984) to be used to assess neutralizing effect against live *P. falciparum* sporozoites. In the ISI assay, the human hepatoma cloned cell line HepG2-A16$_2$ was grown to near confluency on 1% CO_2 glass cover slips in MEM and 10% bovine fetal serum. Antisera or purified antibodies were diluted in culture medium (see table below) and added to the cell cultures. 30,000 *P. falciparum* sporozoites isolated from dissected mosquito salivary glands are counted, diluted and added to each cell culture. The cultures are incubated at 37°C for 2.5 hours, rinsed with PES, and fixed with methanol. Fixed cultures are reacted in an immunoperoxidase antibody assay using a labelled mAb which recognizes the *P. falciparum* CS protein to visualize invaded sporozoites. Then, the number of invading sporozoites are counted by phase microscopy at 400x. The ISI is the percent reduction in invasion in the presence of the test antibody, the humanized antibody, as compared to a control (i.e. non-related) antibody. The assay ranks antibodies in order according to their relative potency.

The following table provides the results performed on the chimeric and synthetic antibodies described previously. Values given are percent inhibition and are the average of 2-3 independent assays.

Summary of ISI Studies

In μg/ml:	20	10	5.0	2.0	1.0	0.1
Chimeric	99	98.5	98	88	83	50
PfHzhc2-3/lc1-1	92	75.5		60		
PfHzhc2-3/lc1-2	85	80.0		0		
PfHzhc2-6/lc1-1		90.0	75		53	0
PfHzhc2-6/lc1-2		87.0	65		50	0

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. For example, recombinant antibodies capable of neutralizing pathogens other than *P. falciparum* may be provided according to the teachings of this invention for the development of prophylactic agents capable of conferring passive immunity to other human diseases. Preferably, engineered antibodies capable of recognizing repeat regions on other malaria pathogens or engineered antibodies to any region on the surface of any stages of the life-cycle of the plasmodium species or capable of neutralizing any stage in the life cycle of the parasite, are desirable starting materials to develop passive immunity agents according to this invention. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SmithKline Beecham, Corporation
U. S. Government, Secretary of the Navy
U. S. Government, Secretary of the Army

(ii) TITLE OF INVENTION: Novel Antibodies for Conferring Passive Immunity Against Infection by a Pathogen in Man

(iii) NUMBER OF SEQUENCES: 61

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Spring House
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19477

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/941,654
(B) FILING DATE: 09-SEP-1992

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(B) REGISTRATION NUMBER: 31,215
(C) REFERENCE/DOCKET NUMBER: SBC P50107

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (215) 540-5818

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn
  1               5                  10                  15

Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
                20                  25                  30

Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn
                35                  40                  45

Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro
                50                  55                  60

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn
                65                  70                  75

Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
                80                  85                  90

Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn
                95                 100                 105

Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro
               110                 115                 120

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn
               125                 130                 135

Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Val
               140                 145                 150

Asp Pro Asn Val Asp Pro Asn Val Asp Pro Asn Val Asp Pro
               155                 160
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 163 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro
 1               5                  10                  15

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn
            20                  25                  30

Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
            35                  40                  45

Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn
            50                  55                  60

Ala Asn Pro Asn Val Asp Pro Asn Ala Asn Pro Asn Ala Asn Pro
            65                  70                  75

Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn
            80                  85                  90

Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
            95                  100                 105

Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn
            110                 115                 120

Ala Asn Pro Asn Ala Asn Pro Asn Val Asp Pro Leu Arg Arg Thr
            125                 130                 135

His Arg Gly Arg His His Arg Arg His Arg Cys Gly Cys Trp Arg
            140                 145                 150

Leu Tyr Arg Arg His His Arg Trp Gly Arg Ser Gly Ser
            155                 160
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GAT ATT CAG CTG ACC CAG TCT CCA TCC TCC CTA GCT GTG TCA      42
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ala Val Ser
  1               5                  10

GTT GGA GAG AAG GTT ACT ATG AGC TGC AAG TCC AGT CAG AGC      84
Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
 15                  20                  25

CTT TTA TAT AGT AGC AAT CAA AAG AAT TAC TTG GCC TGG TAC     126
Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr
     30                  35                  40

CAG CAG AAA CCA GGG CAG TCT CCT AAA CTG CTG ATT TAC TGG     168
Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp
         45                  50                  55

GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC ACA GGC     210
Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
             60                  65                  70

AGA GGA TCC GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT GTG     252
Arg Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val
                 75                  80

AAG GCT GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT     294
Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
 85                  90                  95

AGC TAT CCT CGG ACG TTC GGT GGA GGG ACC AAG CTG GAG ATC     336
Ser Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
    100                 105                 110

AAA                                                        339
Lys
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Val
  1               5                  10                  15
```

```
Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
                  20                  25                  30

Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
                  35                  40                  45

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
                  50                  55                  60

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Arg Gly Ser Gly Thr
                  65                  70                  75

Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala
                  80                  85                  90

Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
                  95                 100                 105

Gly Gly Thr Lys Leu Glu Ile Lys
                 110
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG CTA GCT GTG TCT      42
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser
  1               5                  10

CTG GGC GAG AGG GCC ACC ATC AAC TGC AAG AGC TCT CAG AGC      84
Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser
 15                  20                  25

CTT TTA TAC TCG AGC AAT CAA AAG AAT TAC TTG GCC TGG TAT     126
Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr
     30                  35                  40
```

```
CAG CAG AAA CCC GGG CAG TCT CCT AAG TTG CTC ATT TAC TGG     168
Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp
         45                  50                  55

GCG TCA ACT AGG GAA TCT GGG GTA CCT GAC CGA TTC AGT GGC     210
Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly
             60                  65                  70

AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG     252
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu
                 75                  80

CAG GCT GAA GAT GTG GCA GTA TAC TAC TGT CAG CAA TAT TAT     294
Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
 85                  90                  95

AGC TAT CCG CGG ACG TTC GGC GGA GGG ACC AAG GTG GAG ATC     336
Ser Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
    100                 105                 110

AAA                                                        339
Lys
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
 1               5                  10                  15

Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu
            20                  25                  30

Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
            35                  40                  45

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
            50                  55                  60

Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
            65                  70                  75
```

```
Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala
                 80                  85                  90

Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
                 95                 100                 105

Gly Gly Thr Lys Val Glu Ile Lys
                110
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG CTA GCT GTG TCT      42
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser
  1               5                  10

CTG GGC GAG AGG GCC ACC ATC AAC TGC AAG AGC TCT CAG AGC      84
Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser
 15                  20                  25

CTT TTA TAC TCG AGC AAT CAA AAG AAT TAC TTG GCC TGG TAT     126
Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr
     30                  35                  40

CAG CAG AAA CCC GGG CAG CCT CCT AAG TTG CTC ATT TAC TGG     168
Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp
         45                  50                  55

GCG TCG ACT AGG GAA TCT GGG GTA CCT GAC CGA TTC AGT GGC     210
Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly
             60                  65                  70

AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG     252
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu
                 75                  80
```

```
CAG GCT GAA GAT GTG GCA GTA TAC TAC TGT CAG CAA TAT TAT     294
Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
 85                  90                  95

AGC TAT CCG CGG ACG TTC GGC GGA GGG ACC AAG GTG GAG ATC     336
Ser Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
    100                 105                 110

AAA                                                         339
Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
 1               5                  10                  15

Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu
            20                  25                  30

Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
            35                  40                  45

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
            50                  55                  60

Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
            65                  70                  75

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala
            80                  85                  90

Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
            95                 100                 105

Gly Gly Thr Lys Val Glu Ile Lys
           110
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CTC GAG TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG      42
Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu
  1               5                  10

AAA ATC TCC TGC GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT      84
Lys Ile Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 15                  20                  25

GCC ATG TCT TGG GTT CGC CAG TCT CCA GAG AAG AGG CTG GAG     126
Ala Met Ser Trp Val Arg Gln Ser Pro Glu Lys Arg Leu Glu
     30                  35                  40

TGG GTC GCA GAA ATT AGT GAT GGT GGT AGT TAC ACC TAC TAT     168
Trp Val Ala Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr
         45                  50                  55

CCA GAC ACT GTG ACG GGC CGA TTC ACC ATC TCC AGA GAC AAT     210
Pro Asp Thr Val Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn
             60                  65                  70

GCC AAG AAC ACC CTA TAC CTG GAA ATG AGC AGT CTG AGG TCT     252
Ala Lys Asn Thr Leu Tyr Leu Glu Met Ser Ser Leu Arg Ser
                 75                  80

GAG GAC ACG GCC ATG TAT TAC TGT GCA AGC CTC ATC TAC TAT     294
Glu Asp Thr Ala Met Tyr Tyr Cys Ala Ser Leu Ile Tyr Tyr
 85                  90                  95

GGT TAC GAC GGG TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC     336
Gly Tyr Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
    100                 105                 110

TCA GTC ACC GTC TCC TCA                                    354
Ser Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
 1               5                  10                  15

Ile Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met
            20                  25                  30

Ser Trp Val Arg Gln Ser Pro Glu Lys Arg Leu Glu Trp Val Ala
            35                  40                  45

Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val
            50                  55                  60

Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
            65                  70                  75

Tyr Leu Glu Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr
            80                  85                  90

Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr Asp Gly Tyr Ala Met
            95                  100                 105

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
            110                 115
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 389 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..366

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG CCT      42
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro
  1               5                  10

GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA TTC ACC      84
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
 15                  20                  25

TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG     126
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly
     30                  35                  40

AAA GGT CTA GAG TGG GTC TCA GAA ATT AGT GAT GGT GGT AGT     168
Lys Gly Leu Glu Trp Val Ser Glu Ile Ser Asp Gly Gly Ser
         45                  50                  55

TAC ACC TAC TAT CCA GAC ACT GTG ACG GGC CGG TTC ACG ATA     210
Tyr Thr Tyr Tyr Pro Asp Thr Val Thr Gly Arg Phe Thr Ile
             60                  65                  70

TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC     252
Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn
                 75                  80

AGC CTG AGA GCC GAG GAC ACT GCA GTA TAT TAC TGT GCG AAA     294
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
 85                  90                  95

CTC ATC TAC TAT GGT TAC GAC GGG TAT GCT ATG GAC TAC TGG     336
Leu Ile Tyr Tyr Gly Tyr Asp Gly Tyr Ala Met Asp Tyr Trp
    100                 105                 110

GGC CAG GGT ACC CTG GTC ACC GTG AGC TCA GCTAGTACCA          376
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120

AGGGCCCAAG CTT                                             389
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
            35                  40                  45

Glu Trp Val Ser Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr
            50                  55                  60

Pro Asp Thr Val Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
            65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
            80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Lys Leu Ile Tyr Tyr Gly Tyr Asp
            95                 100                 105

Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
           110                 115                 120

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 389 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..366

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG CCT      42
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro
 1               5                  10

GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA TTC ACC      84
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
 15                  20                  25
```

```
TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG     126
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly
     30                  35                  40

AAA GGT CTA GAG TGG GTC TCA GAA ATT AGT GAT GGT GGT AGT     168
Lys Gly Leu Glu Trp Val Ser Glu Ile Ser Asp Gly Gly Ser
         45                  50                  55

TAC ACC TAC TAT CCA GAC ACT GTG ACG GGC CGG TTC ACG ATA     210
Tyr Thr Tyr Tyr Pro Asp Thr Val Thr Gly Arg Phe Thr Ile
             60                  65                  70

TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC     252
Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn
                 75                  80

AGC CTG AGA GCC GAG GAC ACT GCA GTG TAT TAC TGT GCA TCT     294
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ser
 85                  90                  95

CTC ATC TAC TAT GGT TAC GAC GGG TAT GCT ATG GAC TAC TGG     336
Leu Ile Tyr Tyr Gly Tyr Asp Gly Tyr Ala Met Asp Tyr Trp
    100                 105                 110

GGC CAA GGT ACC CTG GTC ACC GTG AGC TCA GCTAGTACCA         376
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120

AGGGCCCAAG CTT                                            389
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
            35                  40                  45
```

Glu Trp Val Ser Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr
50 55 60

Pro Asp Thr Val Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr Asp
95 100 105

Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCTATGCCA TGAGC 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ser Tyr Ala Met Ser
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAAATTAGTG ATGGTGGTAG TTACACCTAC TATCCAGACA CTGTGACGGG C 51

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr
1 5 10

Val Thr Gly
15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTCATCTACT ATGGTTACGA CGGGTATGCT ATGGACTAC 39

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Ile Tyr Tyr Gly Tyr Asp Gly Tyr Ala Met Asp Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGAGCTCTC AGAGCCTTTT ATACTCGAGC AATCAAAAGA ATTACTTGGC C 51

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn
1 5 10

Tyr Leu Ala
15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TGGGCGTCAA CTAGGGAATC T 21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Trp Ala Ser Thr Arg Glu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGCAATATT ATAGCTATCC GCGGACG 27

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Pro Asn Ala Asn Pro Asn
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCAGATGTAA GCTTCAGCTG ACCCAGTCTC CA 32

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATCTAGATG GCGCCGCCAC AGTACGTTTG ATCTCCAGCT TGGTCCC 47

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGGGTACCAG GTCCARCTKC TCGAGTCWGG 30

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCCTGCAGCT AGCTGAGGAG ACGGTGACCG TGGTCCCTTG GCCCCAG 47

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AGCTATGCCA TGTCT 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Tyr Ala Met Ser
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAGTCCAGTC AGAGCCTTTT ATATAGTAGC AATCAAAAGA ATTACTTGGC C 51

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn
1 5 10

Tyr Leu Ala
15

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGGGCATCCA CTAGGGAATC T 21

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Trp Ala Ser Thr Arg Glu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CAGCAATATT ATAGCTATCC TCGGACG 27

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr
1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGGGCGTCGA CTAGGGAATC T 21

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Trp Ala Ser Thr Arg Glu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 366 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..366

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG CCT      42
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro
  1               5                  10

GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA TTC ACC      84
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
 15                  20                  25

TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG GCT CCA GGG     126
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly
      30                  35                  40

AAA GGT CTA GAG TGG GTC GCA GAG ATC TCT GAT GGT GGT AGT     168
Lys Gly Leu Glu Trp Val Ala Glu Ile Ser Asp Gly Gly Ser
          45                  50                  55

TAC ACC TAC TAT CCA GAC ACT GTG ACG GGC CGG TTC ACG ATA     210
Tyr Thr Tyr Tyr Pro Asp Thr Val Thr Gly Arg Phe Thr Ile
              60                  65                  70

TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC     252
Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn
                  75                  80

AGC CTG AGA GCC GAG GAC ACT GCA GTG TAT TAC TGT GCA TCT     294
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ser
 85                  90                  95

CTC ATC TAC TAT GGT TAC GAC GGG TAT GCT ATG GAC TAC TGG     336
Leu Ile Tyr Tyr Gly Tyr Asp Gly Tyr Ala Met Asp Tyr Trp
    100                 105                 110

GGC CAA GGT ACC CTG GTC ACC GTG AGC TCA                     366
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
            35                  40                  45

Glu Trp Val Ala Glu Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr
            50                  55                  60

Pro Asp Thr Val Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
            65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
            80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr Asp
            95                 100                 105

Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
           110                 115                 120

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TAGTGAAGAA TTCGAGGACG CCAGCAACAT GGTGTTGCAG ACCCAGGTCT 50

TCATTTCTCT GTTGCTCTGG ATCTCTGGTG CCTACGGGGA GGTGCAG 97

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCTAGCGGAT TCACCTTTAG CAGCCATGTC GGACCCCCCA GGGACTCTGA 50

GAGGACACGT CGATCGCCTA AGTGGAAATC CTATGCCATG AGCTGGGTCC 100

GCCAGGCTCC AGGGAAAGGT CTAGAGTGGG TCTCAGAAAT CTTTATAGTG 150

ATGGTGGTAG TTAC 164

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGTCTC 50

TGTTAAGGTT CTTGTGCGAC ATAGACGTTT ACTGCAGTAT ATTACTGTGC 100

GAAACTCATC TACTATGGTT ACGACGGGTA TGCTATGGAC TAGCTGCCCA 150

TACGATACCT GATC 164

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTCTTGAAAG CTTGGGCCCT TGGTACTAGC TGAGCTCACG GTGACCAGGG 50

TACCCTGGCC CCAGTAGTCC ATAGCATACC CGTCG 85

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CATTTGCAGA TACAGCGTGT TCTTGGAATT GTCTCTGGAT ATCGTGAACC 50

GGCCCGTCAC AGTGTCTGGA TAGTAGGTGT AACTACCACC ATCACTAATT TC 102

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CTAAAGGTGA ATCCGCTAGC TGCACAGGAG AGTCTCAGGG ACCCCCCAGG 50

CTGTACCAAG CCTCCCCCAG ACTCGAGCAG CTGCACCTCC CCGTAGGCAC C 101

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCGCGAATTC GAGGACGCCA GCAAC 25

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CCGCAAGCTT GGGCCCTTGG TACTAGCT 28

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TAAGCGGAAT TCGTAGTCGG ATATCGTGAT GACCCAGTCT CCAGACTCGC 50

TAGCTGTGTC TCTGGGCGAG AGGGC 75

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TTACTTGGCC TGGTATCAGC AGAAACCCGG GCAGTCTCCT AAGTTGCTCA 50

TAGTTTTCTT AATGAACCGG ACTTACTGGG CGTCAACTAG 90

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GACAGATTTC ACTCTCACCA TCAGCAGCCT GCAGGCTGAA GATGTGGCAG 50

TATACTACTG CTGTCTAAAG TGTCAGCAAT ATTATAGCTA TCC 93

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CAGTTGGAAA GCTTGGCGCC GCCACAGTAC GTTTGATCTC CACCTTGGTC 50

CCTCCGCCGA ACGTCCGCGG ATAGCTATAA TATTGC 86

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GTGAAATCTG TCCCAGACCC GCTGCCACTG AATCGGTCAG GTACCCCAGA 50

TTCCCTAGTT GACGCC 66

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CAGGCCAAGT AATTCTTTTG ATTGCTCGAG TATAAAAGGC TCTGAGAGCT 50

CTTGCAGTTG ATGGTGGCCC TCTCGCCC 78

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GCGGAATTCG TAGTCGGATA TCGTGATGAC 30

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TGGAAAGCTT GGCGCCGCCA CAGTACGTTT GATC 34

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..57

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
ATG GTG TTG CAG ACC CAG GTC TTC ATT TCT CTG TTG CTC TGG      42
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp
  1               5                  10

ATC TCT GGT GCC TAC                                        57
Ile Ser Gly Ala Tyr
 15
```

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile
1 5 10 15

Ser Gly Ala Tyr

WHAT IS CLAIMED IS:

1. A fusion molecule comprising a first fusion partner nucleotide sequence encoding an amino acid sequence having the antigen specificity of a anti-*Plasmodium* antibody, operatively linked in frame to a second fusion partner nucleotide sequence.

2. The molecule according to claim 1 wherein said first fusion partner is a synthetic immunoglobulin variable region nucleotide sequence encoding an amino acid sequence comprising a complementarity determining region originating from a *Plasmodium* antibody, a fragment or allelic variation or modification thereof.

3. The molecule according to claim 2 wherein said second fusion partner is a heterologous immunoglobulin variable framework region.

4. The molecule according to claim 2 wherein said variable region nucleotide sequence is selected from the group consisting of a heavy chain variable region and a light chain variable region.

5. The molecule according to claim 4 selected from the group consisting of

(a) a heavy chain nucleotide sequence of Fig. 5 (SEQ ID NO: 11);

(b) a heavy chain nucleotide sequence of Fig. 6 (SEQ ID NO: 13);

(c) a light chain nucleotide sequence of Fig. 2 (SEQ ID NO: 5) and Fig. 3 (SEQ ID NO: 7).

(d) a light chain nucleotide sequence of Fig. 3 (SEQ ID NO: 7); and

(e) functional fragments thereof.

6. The molecule according to claim 4 wherein said first fusion partner nucleotide sequence comprises a sequence selected from the group consisting of

(a) AGCTATGCCATGAGC: SEQ ID NO: 15;

(b) GAAATTAGTGATGGTGGTAGTTACACCTACTATCCA GACACTGTGACGGGC: SEQ ID NO: 17;

(c) CTCATCTACTATGGTTACGACGGGTATGCTATGGAC TAC: SEQ ID NO: 19;

(d) AAGAGCTCTCAGAGCCTTTTATACTCGAGCAATCAA AAGAATTACTTGGCC: SEQ ID NO: 21;

(e) TGGGCGTCAACTAGGGAATCT: SEQ ID NO: 23;

(f) CAGCAATATTATAGCTATCCGCGGACG: SEQ ID NO: 25;

(g) AGCTATGCCATGTCT: SEQ ID NO: 32;

(h) AAGTCCAGTCAGAGCCTTTTATATAGTAGCAATCAAA AGAATTACTTGGCC: SEQ ID NO: 34;

(i) TGGGCATCCACTAGGGAATCT: SEQ ID NO: 36;

(j) CAGCAATATTATAGCTATCCTCGGACG: SEQ ID NO: 38;

(k) TGGGCGTCGACTAGGGAATCT: SEQ ID NO: 41;

and an allelic variation or modification thereof, characterized by the antigen specificity of murine NFS2, said nucleic acid sequence optionally containing restriction sites to facilitate insertion into a desired antibody framework region or a plasmid vector.

7. A synthetic immunoglobulin variable region nucleotide sequence encoding an amino acid sequence comprising a complementarity determining region originating from a *Plasmodium* antibody, a fragment or allelic variation or modification thereof.

8. A fusion protein comprising a first amino acid sequence derived from a *Plasmodium* antibody capable of binding an epitope on a selected *Plasmodium* species, said sequence having the antigen specificity of said antibody fused to a heterologous second amino acid sequence.

9. The fusion protein according to claim 8 wherein said first amino acid sequence comprises an amino acid sequence selected from the group consisting of:

(a) a variable heavy chain sequence of said antibody;

(b) a variable light chain sequence of said antibody;

(c) a complementarity determining region of said antibody; and

(d) a functional fragment of (a) through (c).

10. The fusion protein according to claim 8 wherein said fusion protein is selected from the group consisting of

(a) a complete engineered antibody, having full length heavy and light chains comprising at least fragments of the variable regions derived from said *Plasmodium* antibody;

(b) the F_{ab} or $(F_{ab}')_2$ fragment of the engineered antibody of (a);

(c) a dimer formed of heavy chains derived from the engineered antibody of (a);

(d) an F_v fragment of the engineered antibody of (a); and

(e) a single-chain antibody derived from the engineered antibody of (a);

said protein having the same specificity as said *Plasmodium* antibody.

11. An engineered *P. falciparum* antibody comprising a heavy chain comprising a complementarity determining region derived from the variable heavy chain region of a non-human *P. falciparum* monoclonal antibody.

12. The antibody according to claim 11 wherein said non-human CDRs are in operative association with one of the group consisting of

(a) a selected human antibody heavy chain framework and constant regions; and

(b) the heavy chain framework from said antibody and a constant region from a selected human antibody.

13. The antibody according to claim 11 further comprising a light chain selected from the group consisting of

(a) a light chain comprising a CDR derived from the variable light chain region of said monoclonal antibody in operative association with selected human antibody light chain framework and constant regions;

(b) the light chain framework from said antibody and a constant region from a selected human antibody;

(c) the complete light chain from said anti-*Plasmodium* antibody; and

(d) the complete light chain from a selected human antibody.

14. The antibody according to claim 11, wherein said heavy chain comprises a variable heavy chain sequence selected from the sequences of Fig. 5 (SEQ ID NO: 12), Fig. 6 (SEQ ID NO: 14), Pfhzhc2-3 (SEQ ID NO:14), and Pfhzhc2-6 (SEQ ID NO:42).

15. The antibody according to claim 13 wherein said light chain comprises a variable light chain sequence selected from the sequences of Fig. 2 (SEQ ID NO: 6) and Fig. 3 (SEQ ID NO: 8).

16. The antibody according to claim 13 wherein light chain complementarity determining region is selected from one or more of the sequences consisting of

(a) LysSerSerGlnSerLeuLeuTyrSerSerAsn GlnLysAsnTyrLeuAla: SEQ ID NO: 22;

(b) TrpAlaSerThrArgGluSer: SEQ ID NO: 24; and

(c) GlnGlnTyrTyrSerTyrProArgThr: SEQ ID NO: 26.

17. The antibody according to claim 11 wherein said heavy chain complementarity determining region is selected from one or more of the sequences consisting of

(a) SerTyrAlaMetSer: SEQ ID NO: 16;

(b) GluIleSerAspGlyGlySerTyrThrTyrTyrPro AspThrValThrGly: SEQ ID NO: 18; and

(c) LeuIleTyrTyrGlyTyrAspGlyTyrAlaMet AspTyr: SEQ ID NO: 20.

18. An anti-*P. falciparum* complementarity determining region peptide selected from the group consisting of

(a) SerTyrAlaMetSer: SEQ ID NO: 16;

(b) GluIleSerAspGlyGlySerTyrThrTyrTyr ProAspThrValThrGly: SEQ ID NO: 18;

(c) LeuIleTyrTyrGlyTyrAspGlyTyrAlaMet AspTyr: SEQ ID NO: 20;

(d) LysSerSerGlnSerLeuLeuTyrSerSerAsn GlnLysAsnTyrLeuAla: SEQ ID NO: 22;

(e) TrpAlaSerThrArgGluSer: SEQ ID NO: 24;

(f) GlnGlnTyrTyrSerTyrProArgThr: SEQ ID NO: 26;

and an analog thereof, characterized by the antigen specificity of NFS2.

19. A synthetic immunoglobulin variable chain amino acid sequence comprising a complementarity determining region originating from a *Plasmodium* antibody in a heterologous variable chain framework, a fragment or analog thereof sharing the anti-*Plasmodium* antigen specificity of said sequence.

20. The sequence according to claim 19 selected from the group consisting of the amino acid sequences of Fig. 5 (SEQ ID NO: 12), Fig. 6 (SEQ ID NO: 14), Fig. 2 (SEQ ID NO: 6) and Fig. 3 (SEQ ID NO: 8).

21. A monoclonal antibody, other than NFS2, which is capable of binding to a *P. falciparum* epitope comprising the sequence Pro Asn Ala Asn Pro Asn SEQ ID NO: 27, a F_{ab} fragment thereof, or a $(F_{ab}')_2$ fragment thereof.

22. A pharmaceutical prophylactic composition comprising a fusion protein or antibody according to any of claims 8 through 21 and a pharmaceutically acceptable carrier or diluent.

23. A pharmaceutical composition according to claim 22 wherein said protein is a humanized *P. falciparum* antibody.

24. A recombinant plasmid comprising a nucleic acid sequence of any of claims 1 through 7 in operative association with a regulatory control sequence capable of directing the replication and expression of said nucleic acid sequence in a selected host cell.

25. A mammalian cell line transfected with at least one recombinant plasmid comprising a nucleic acid sequence of any of claims 1 through 7.

26. A method of producing a engineered antibody comprising culturing a mammalian cell line transfected with at least one recombinant plasmid comprising a nucleic acid sequence of any of claims 1 through 7 under suitable conditions permitting expression and assembly of complementary heavy and light chains, and recovering the assembled antibody from the cell culture.

27. The use of a protein or antibody of claims 8 through 21 in the preparation of a pharmaceutical composition suitable for passively protecting a human against infection by a *Plasmodium* species.

FIGURE 1

```
GAT ATT CAG CTG ACC CAG TCT CCA TCC TCC CTA  33
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu
  1               5                  10

GCT GTG TCA GTT GGA GAG AAG GTT ACT ATG AGC  66
Ala Val Ser Val Gly Glu Lys Val Thr Met Ser
            15                  20

TGC AAG TCC AGT CAG AGC CTT TTA TAT AGT AGC  99
Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser
        25                  30

AAT CAA AAG AAT TAC TTG GCC TGG TAC CAG CAG 132
Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
    35                  40

AAA CCA GGG CAG TCT CCT AAA CTG CTG ATT TAC 165
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
45                  50                  55

TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT 198
Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp
                60                  65

CGC TTC ACA GGC AGA GGA TCC GGG ACA GAT TTC 231
Arg Phe Thr Gly Arg Gly Ser Gly Thr Asp Phe
            70                  75

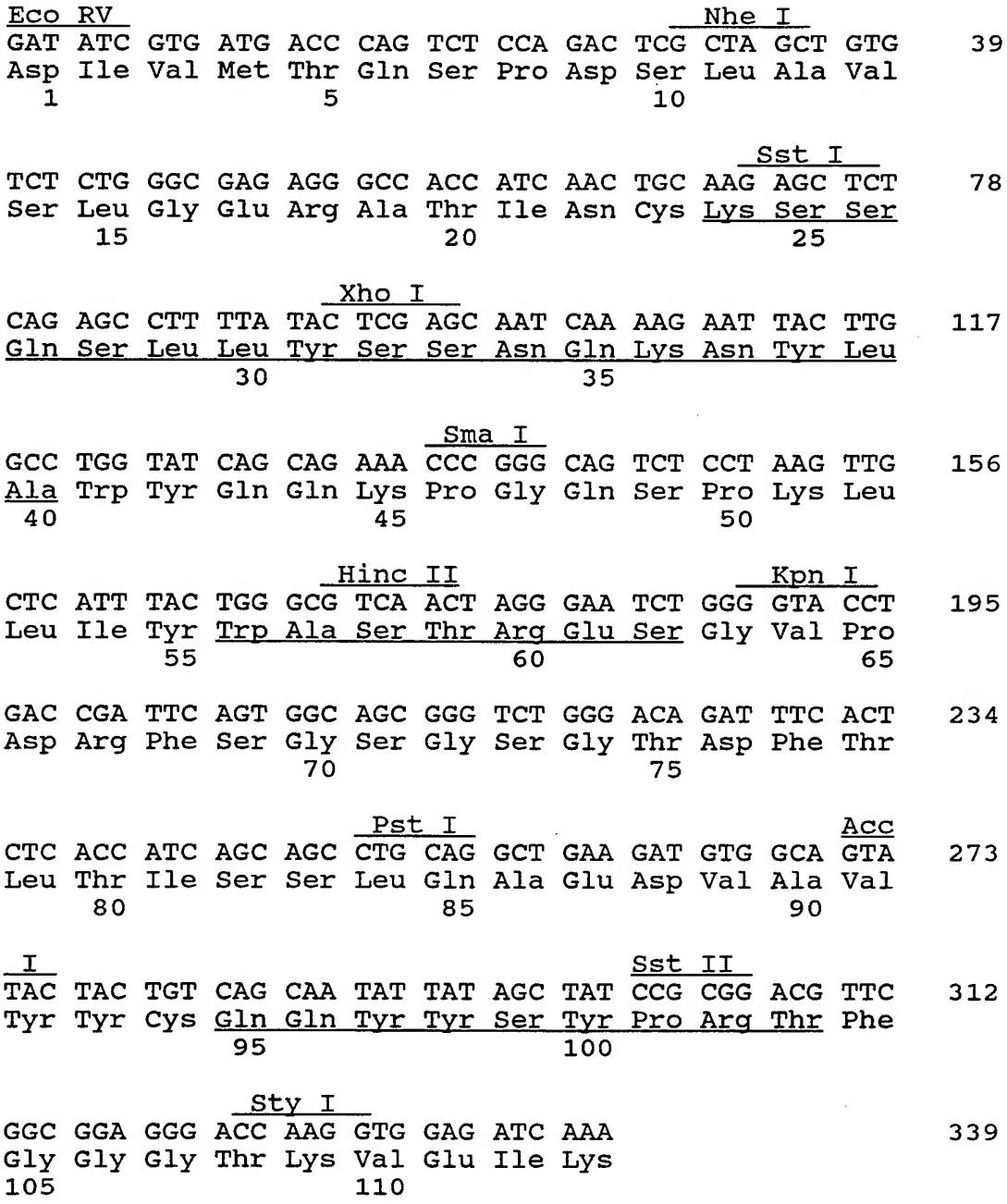
ACT CTC ACC ATC AGC AGT GTG AAG GCT GAA GAC 264
Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp
        80                  85

CTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGC 297
Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser
    90                  95

TAT CCT CGG ACG TTC GGT GGA GGG ACC AAG CTG 330
Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu
100                 105                 110

GAG ATC AAA                             339
Glu Ile Lys
```

Figure 2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Nhe I
GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG CTA GCT GTG 39
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val
1 5 10
Sst I
TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC AAG AGC TCT 78
Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser
15 20 25
Xho I
CAG AGC CTT TTA TAC TCG AGC AAT CAA AAG AAT TAC TTG 117
Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
30 35
Sma I
GCC TGG TAT CAG CAG AAA CCC GGG CAG TCT CCT AAG TTG 156
Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu
40 45 50
Hinc II
Kpn I
CTC ATT TAC TGG GCG TCA ACT AGG GAA TCT GGG GTA CCT 195
Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro
55 60 65
GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT 234
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
70 75
Pst I
Acc
CTC ACC ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA GTA 273
Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
80 85 90
I
Sst II
TAC TAC TGT CAG CAA TAT TAT AGC TAT CCG CGG ACG TTC 312
Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe
95 100
Sty I
GGC GGA GGG ACC AAG GTG GAG ATC AAA 339
Gly Gly Gly Thr Lys Val Glu Ile Lys
105 110

Figure 3

```
Eco RV                                                    Nhe I
GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG CTA GCT GTG          39
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val
  1               5                  10

                                                     Sst I
TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC AAG AGC TCT          78
Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser
     15                  20                  25

                        Xho I
CAG AGC CTT TTA TAC TCG AGC AAT CAA AAG AAT TAC TTG         117
Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
               30                  35

                              Sma I
GCC TGG TAT CAG CAG AAA CCC GGG CAG CCT CCT AAG TTG         156
Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu
 40                  45                  50

                    Hinc II                          Kpn I
CTC ATT TAC TGG GCG TCG ACT AGG GAA TCT GGG GTA CCT         195
Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro
         55                  60                  65

GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT         234
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                70                  75

                         Pst I                           Acc
CTC ACC ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA GTA         273
Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
     80                  85                  90

 I                                        Sst II
TAC TAC TGT CAG CAA TAT TAT AGC TAT CCG CGG ACG TTC         312
Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe
               95                  100

               Sty I
GGC GGA GGG ACC AAG GTG GAG ATC AAA                        339
Gly Gly Gly Thr Lys Val Glu Ile Lys
105                 110
```

FIGURE 4

```
CTC GAG TCT GGG GGA GGC TTA GTG AAG CCT   30
Leu Glu Ser Gly Gly Gly Leu Val Lys Pro
  1               5                  10

GGA GGG TCC CTG AAA ATC TCC TGC GCA GCC   60
Gly Gly Ser Leu Lys Ile Ser Cys Ala Ala
                15                  20

TCT GGA TTC ACT TTC AGT AGC TAT GCC ATG   90
Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met
                25                  30

TCT TGG GTT CGC CAG TCT CCA GAG AAG AGG  120
Ser Trp Val Arg Gln Ser Pro Glu Lys Arg
                35                  40

CTG GAG TGG GTC GCA GAA ATT AGT GAT GGT  150
Leu Glu Trp Val Ala Glu Ile Ser Asp Gly
                45                  50

GGT AGT TAC ACC TAC TAT CCA GAC ACT GTG  180
Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val
                55                  60

ACG GGC CGA TTC ACC ATC TCC AGA GAC AAT  210
Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn
                65                  70

GCC AAG AAC ACC CTA TAC CTG GAA ATG AGC  240
Ala Lys Asn Thr Leu Tyr Leu Glu Met Ser
                75                  80

AGT CTG AGG TCT GAG GAC ACG GCC ATG TAT  270
Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr
                85                  90

TAC TGT GCA AGC CTC ATC TAC TAT GGT TAC  300
Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr
                95                 100

GAC GGG TAT GCT ATG GAC TAC TGG GGT CAA  330
Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln
               105                 110

GGA ACC TCA GTC ACC GTC TCC TCA          354
Gly Thr Ser Val Thr Val Ser Ser
               115
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Figure 5

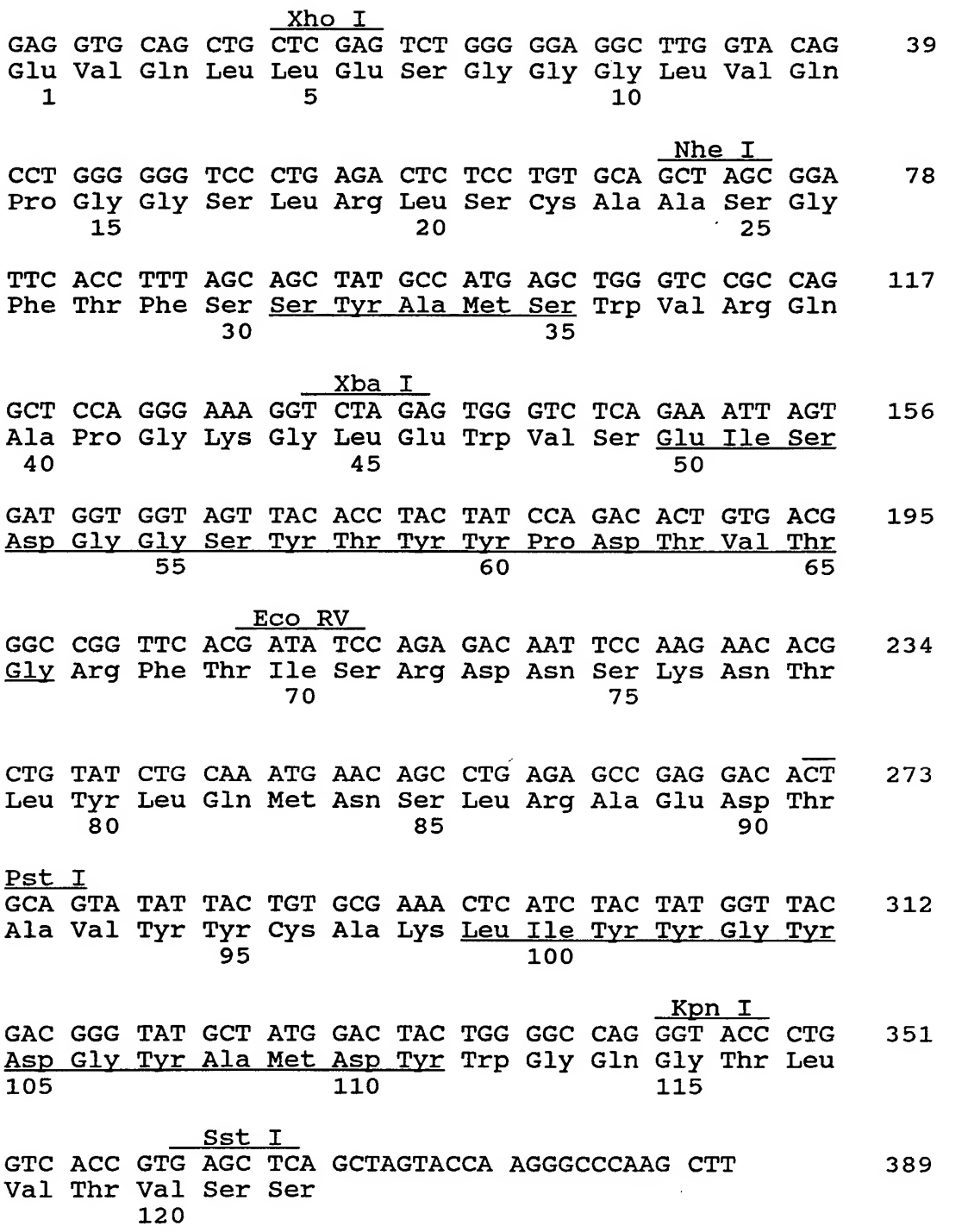
Xho I
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG 39
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
1 5 10
Nhe I
CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA 78
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
15 20 25
TTC ACC TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG 117
Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln
30 35
Xba I
GCT CCA GGG AAA GGT CTA GAG TGG GTC TCA GAA ATT AGT 156
Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Ser
40 45 50
GAT GGT GGT AGT TAC ACC TAC TAT CCA GAC ACT GTG ACG 195
Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val Thr
55 60 65
Eco RV
GGC CGG TTC ACG ATA TCC AGA GAC AAT TCC AAG AAC ACG 234
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
70 75
CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACT 273
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
80 85 90
Pst I
GCA GTA TAT TAC TGT GCG AAA CTC ATC TAC TAT GGT TAC 312
Ala Val Tyr Tyr Cys Ala Lys Leu Ile Tyr Tyr Gly Tyr
95 100
Kpn I
GAC GGG TAT GCT ATG GAC TAC TGG GGC CAG GGT ACC CTG 351
Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
105 110 115
Sst I
GTC ACC GTG AGC TCA GCTAGTACCA AGGGCCCAAG CTT 389
Val Thr Val Ser Ser
120

Figure 6

```
                    Xho I
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG     39
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
  1               5                  10

                                            Nhe I
CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA     78
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
     15                  20                  25

TTC ACC TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG    117
Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln
             30                  35

                      Xba I
GCT CCA GGG AAA GGT CTA GAG TGG GTC TCA GAA ATT AGT    156
Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Ser
 40                  45                  50

GAT GGT GGT AGT TAC ACC TAC TAT CCA GAC ACT GTG ACG    195
Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val Thr
          55                  60                  65

                  Eco RV
GGC CGG TTC ACG ATA TCC AGA GAC AAT TCC AAG AAC ACG    234
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
             70                  75

CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACT    273
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
     80                  85                  90

Pst I
GCA GTG TAT TAC TGT GCA TCT CTC ATC TAC TAT GGT TAC    312
Ala Val Tyr Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr
             95                  100

                                        Kpn I
GAC GGG TAT GCT ATG GAC TAC TGG GGC CAA GGT ACC CTG    351
Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
105                 110                 115

          Sst I
GTC ACC GTG AGC TCA GCTAGTACCA AGGGCCCAAG CTT          389
Val Thr Val Ser Ser
        120
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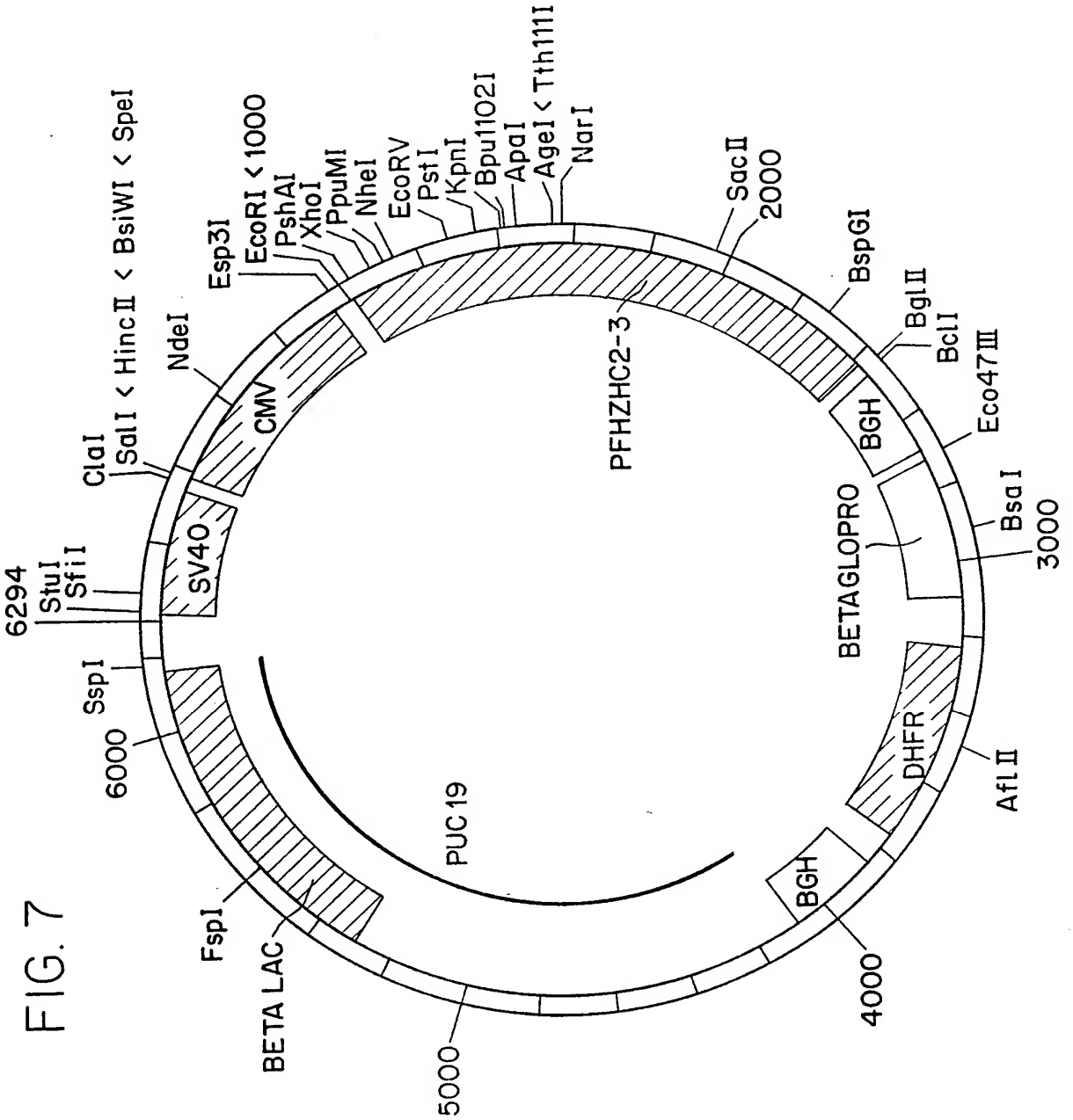
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BspGI
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BclI
Eco47III
BsaI
3000
NdeI
CMV
PFHZHC2-3
BGH
BETAGLOPRO
ClaI
SalI < HincII < BsiWI < SpeI
SV40
6294
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DHFR
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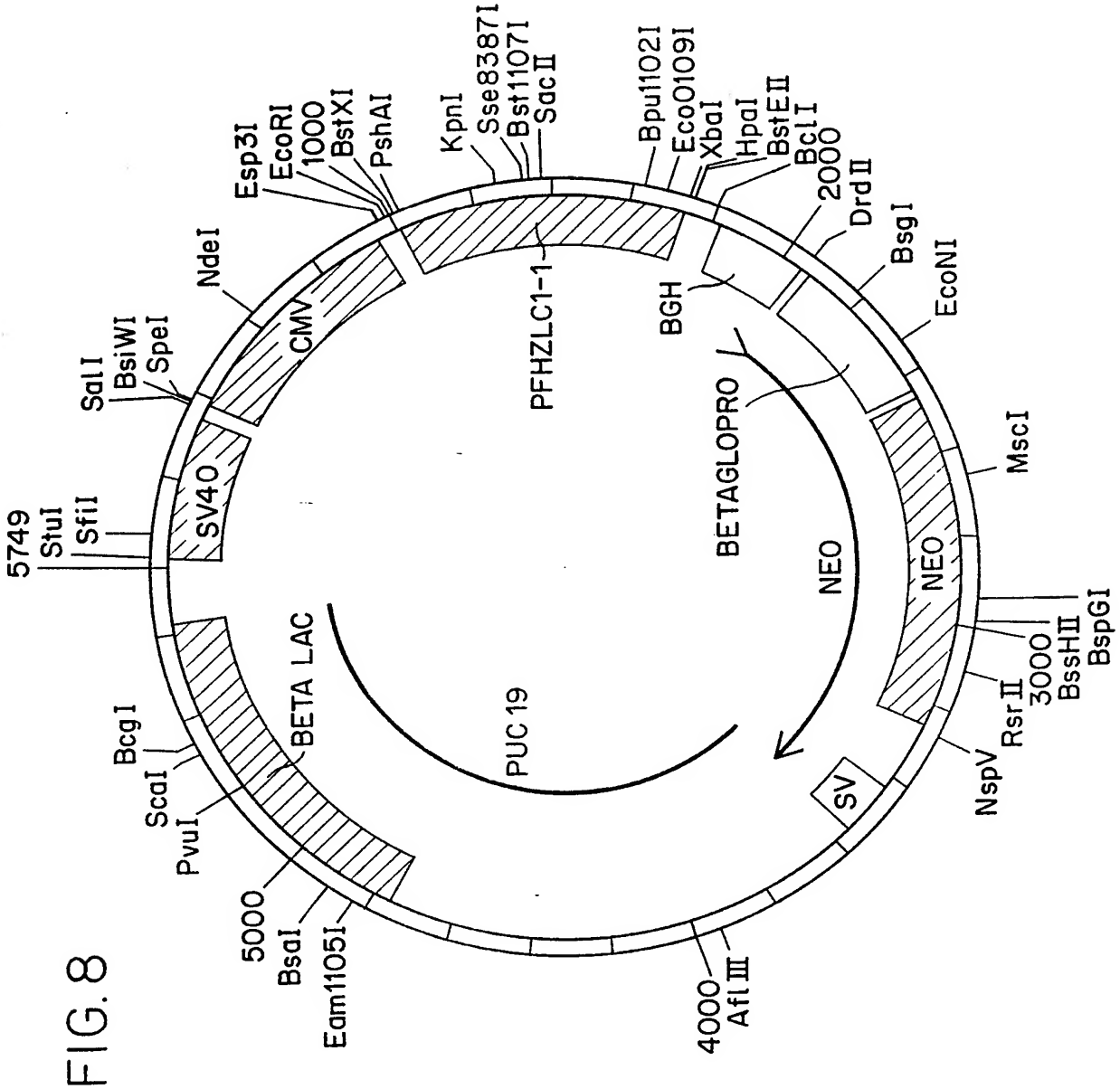
PUC 19
FspI
BETA LAC
BGH
5000
4000

FIG. 7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StuI
SfiI
SalI
BsiWI
SpeI
NdeI
Esp3I
EcoRI
1000
BstXI
PshAI
KpnI
Sse8387I
Bst1107I
SacII
Bpu1102I
Eco0109I
XbaI
HpaI
BstEII
BclI
2000
DrdII
BsgI
EcoNI
MscI
BspGI
BssHII
3000
RsrII
NspV
SV
4000
AflIII
Eam1105I
BsaI
5000
PvuI
ScaI
BcgI
BETA LAC
PUC 19
SV40
CMV
PFHZLC1-1
BGH
BETAGLOPRO
NEO
NEO

FIG. 8

FIGURE 9

```
GAG GTG CAG CTG CTC GAG TCT GGG GGA GGC TTG GTA CAG     39
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
  1               5                  10

CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA GCT AGC GGA     78
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
     15                  20                  25

TTC ACC TTT AGC AGC TAT GCC ATG AGC TGG GTC CGC CAG    117
Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln
             30                  35

GCT CCA GGG AAA GGT CTA GAG TGG GTC GCA GAG ATC TCT    156
Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Glu Ile Ser
 40                  45                  50

GAT GGT GGT AGT TAC ACC TAC TAT CCA GAC ACT GTG ACG    195
Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Thr Val Thr
         55                  60                  65

GGC CGG TTC ACG ATA TCC AGA GAC AAT TCC AAG AAC ACG    234
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
                 70                  75

CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACT    273
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
     80                  85                  90

GCA GTG TAT TAC TGT GCA TCT CTC ATC TAC TAT GGT TAC    312
Ala Val Tyr Tyr Cys Ala Ser Leu Ile Tyr Tyr Gly Tyr
             95                  100

GAC GGG TAT GCT ATG GAC TAC TGG GGC CAA GGT ACC CTG    351
Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
105                 110                 115

GTC ACC GTG AGC TCA                                   366
Val Thr Val Ser Ser
         120
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08435

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :435/240.2, 320.1, 240.27; 424/85.8; 530/387.1, 388.6.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.2, 320.1, 240.27; 424/85.8; 530/387.1, 388.6.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, Biosis, Medline.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, O, 270,077 (Nakatani et. al.) 06 August 1988, see entire document.	6,16-18,25,26
X	The EMBO Journal, Volume 5, No. 7, issued 1986, Andrew J. Caton et. al., "Structural and functional implications of a restricted antibody response to a defined antigenic region on the influenza virus hemagglutinin", pages 1577-1587, see figures 4 and 5.	6, 16-18, 25,26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 December 1993	Date of mailing of the international search report 27 DEC 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer PAULA HUTZELL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08435

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal of Immunology, Volume 139, No.7, issued 01 October 1987, Harout Dersimonian et. al, "Relationship of human variable region heavy chain germ-line genes to genes encoding anti-DNA autoantibodies", pages 2496-2501, see page 2498.	1-27
Y	Bull World Health Organ, 68 Suppl. issued 1990, Mellouk et. al. "Evaluation of an in vitro assay aimed at measuring protective antibodies against sporozoites", pages 52-59, see entire abstract.	1-27
Y	Proceedings of the National Academy of Sciences, Volume 86, issued December 1989, Queen et. al. "A humanized antibody that binds to the interleukin 2 receptor", pages 10029-10033, see entire document.	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08435

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C07H 21/02, 21/04; C12N 15/70, 15/74, 15/79, 5/10; C07K 15/28; A61K 39/395.